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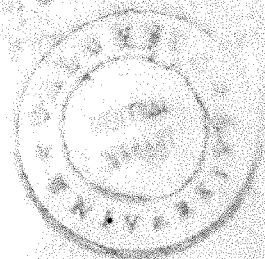
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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Walleto™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.



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BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)

NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)

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ORAL CONTRACEPTIVE (OC) AGENTS

Indications: Prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM:** Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction, b. known or suspected carcinoma of the breast, c. known or suspected estrogen dependent neoplasia, d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes a day) and is particularly marked among women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated a higher risk of non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls, an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CERE-BROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies; in an American study of cerebrovascular disease, diabetes, history of arterial disease, and cigarette smoking, the relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4.95 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of arterial disease, and cigarette smoking) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal MI compared to nonsmoking nonusers, and a 10-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. **RISK OF DOSE.** Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 51 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. **PERSISTENCE OF RISK.** Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI. Another study suggested persistence of risk for subarachnoid hemorrhage. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES.** A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 in ages 15-34, 50/100,000 in ages 35-44, and 140/100,000 in ages 45-49. Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—16/100,000 for nonusers and 1/2000 for smokers, ages 45 and over—1/2500 for nonusers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for pregnancy in women over 40 who smoke. (Rate given for pill only smokers for each age group for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers

(less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of pre-eclampsia toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS - RISK ASSOCIATED WITH USE OF OCs				
Age	Below 30	30-39	40+	
Heavy smokers	A	B	A	
Light smokers	D	C	A	
Nonsmokers	(no predisposing conditions)	D	C	B
Nonsmokers	(other predisposing conditions)	C	B	B
A—Use associated with very high risk				
B—Use associated with high risk				
C—Use associated with moderate risk				
D—Use associated with low risk				

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC use immediately. A 4-6 fold increased risk of post-surgical thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgical thromboembolic complications with contraceptive needs. Data suggest various veins substantially increased risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. **Ocular Lesions:** Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3. **Carcinoma:** Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (13-19 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polyps, cysts, etc.), nearly all occurred in women who had used OCs. OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggested an increased incidence of uterine tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix in long-term (over 10 years) OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In case of abnormal vaginal bleeding, if there is no reported abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. **Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. Several reports have associated benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular carcinoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intrahepatic hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans and biopsy, if done, may be helpful. Liver biopsy may be useful in diagnosing primary liver neoplasms. 5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol (DES) during pregnancy have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4-7 fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data support risk of limb-reduction defects in exposed fetuses and newborns. 6. **Birth Defects:** In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 182/1000 births, compared to 78/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects in women exposed to sex hormones (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency

of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding or to prevent pregnancy. 7. **Gall Bladder Disease:** Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. **Carbohydrate and Lipid Metabolic Effects:** Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, preexisting diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. **Elevated Blood Pressure:** An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the first year of use is 2-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. **Headache:** Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache. Headache is recurrent, persistent, or severe. 10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy. If pregnancy has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become anovulatory after discontinuation of OCs. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary tumor-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. **Fertility:** There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results of pregnancies achieved with OC and other methods. 12. **Discontinuation of Birth Control:** For parous women, the difference is not apparent 30 months after cessation of contraception. 12. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. In combined OCs, the ratio is not different. 13. **Postpartum Period:** Breastfeeding may be affected by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. 14. **Precautions:** GENERAL. 1. Take a complete medical and family history before starting OCs. 2. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Observe patients with a history of uterine leiomyomata. 4. OCs may cause depression or a serious depressive episode. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, heart failure, renal insufficiency, or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may interfere in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of the use of OCs. 10. OCs may be submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin. c. Increased norepinephrine-induced platelet aggregability. d. Increased thyroid binding globulin (TBG) leading to increased circulating free thyroid hormone. As measured by a competitive protein-binding assay (CPBA), or by T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnane diol excretion. e. Reduced response to metoprolol test. f. Increased phospholipids and triglycerides. g. Temporally decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist for a temporary or permanent cessation of wear. 12. **DRUG INTERACTIONS:** OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquillisants, antipsychotics, anticonvulsants, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensives agents (e.g., guanethidine), vitamins, hypoglycemics, tranquilizers, hypnotics, prokinetics, theophylline, and beta blockers. **CARCINOGENESIS:** See Warnings. **PREGNANCY:** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS:** See Warnings. **Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses, during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, mental depression, hirsutism, loss of hair, alopecia, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant neoplasms (hemolytic uremic syndrome), malignant pheochromocytoma, acute renal failure. **Information for the Patient:** (See Patient Package Insert).

Rev. 8/81

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SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera[®] 10 MG TABLETS (medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. 2. Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. 3. Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. 4. Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. 5. Usage in pregnancy is not recommended (see Warning box). 6. Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influ-

enced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginam, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: **Pregnancy:** (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T₃ uptake values).

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For vulvovaginal candidiasis...

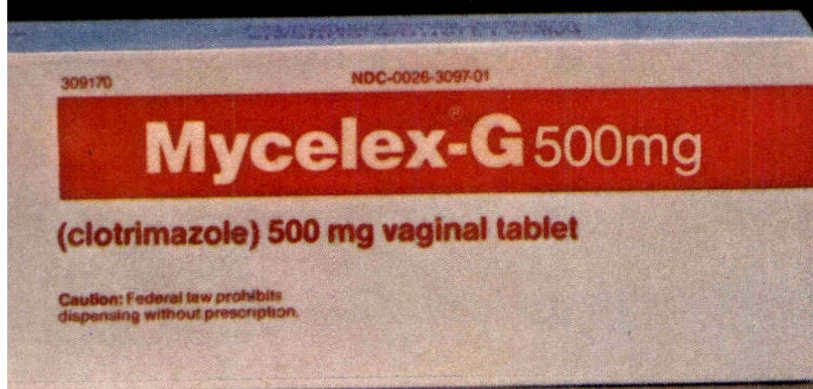
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CONTRAINDICATIONS: Mycelex-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

WARNINGS: None.

PRECAUTIONS: If there is a lack of response to Mycelex-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

CARCINOGENESIS: No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

USAGE IN PREGNANCY: PREGNANCY CATEGORY B: The disposition of ¹⁴C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

ADVERSE REACTIONS: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

OVERDOSAGE: No data available.

DRUG ABUSE AND DEPENDENCE: Drug abuse and dependence with Mycelex-G 500 mg Vaginal Tablets has not been reported.

DOSE AND ADMINISTRATION: The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

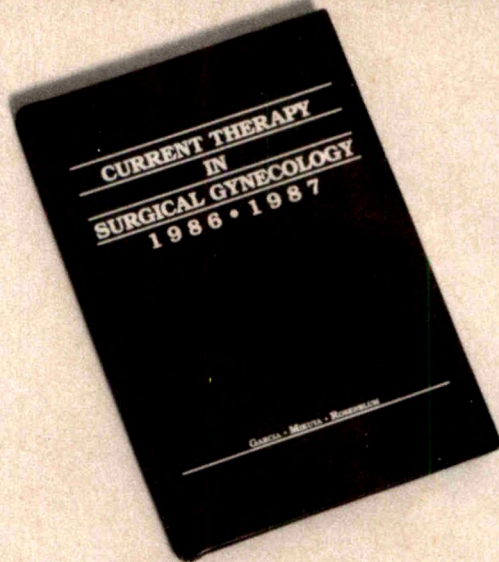
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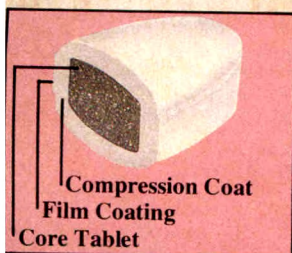
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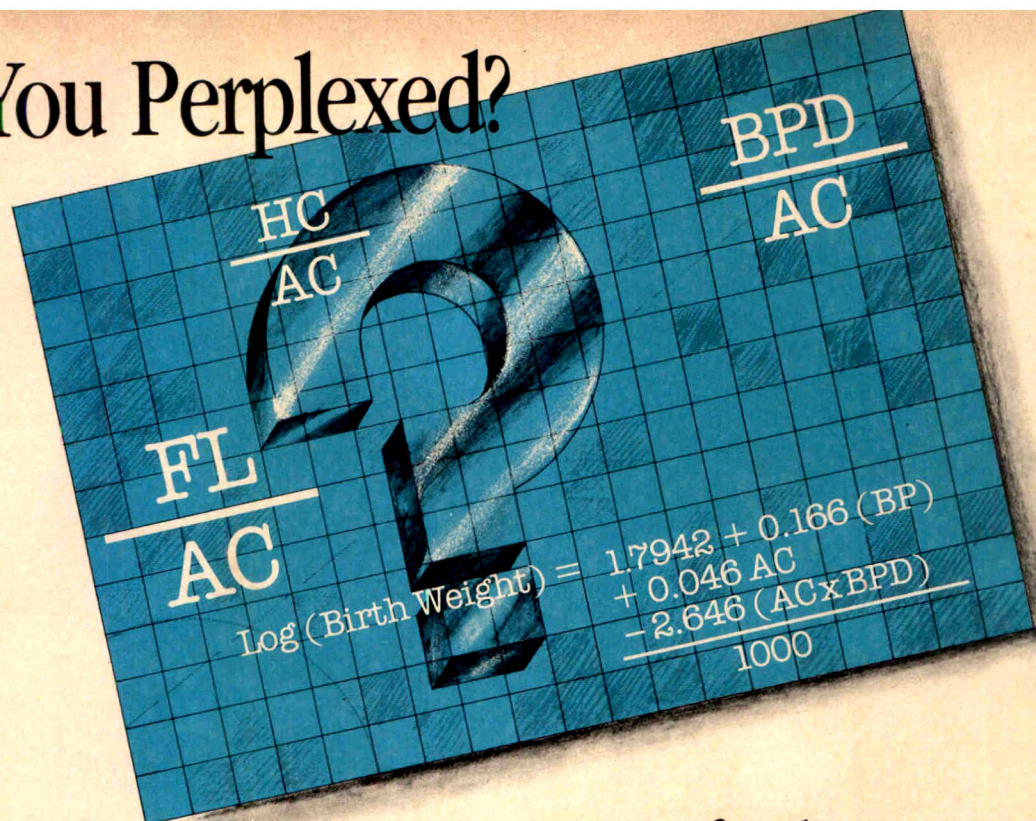
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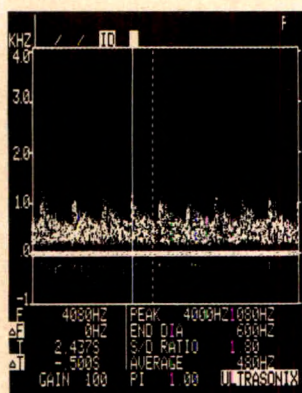
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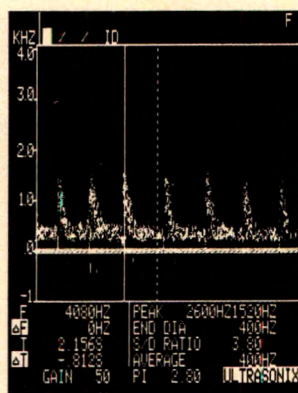
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Why risk significant *Bacteroides fragilis*

Cefotan has gaps* in its coverage of these *B. fragilis* group organisms.**

MEFOXIN does not.

Clinical indications for intraabdominal infections [†]		
	Cefotan^{††} (céfotetan disodium)	Mefoxin[®] (Cefoxitin Sodium MSD)
<i>Bacteroides</i> species		
including <i>B. fragilis</i> [‡]		
<i>B. distasonis</i>		
<i>B. ovatus</i>		
<i>B. thetaiotaomicron</i>		
<i>B. vulgatus</i> [‡]		

[†]MEFOXIN is indicated for the treatment of intraabdominal infections, including peritonitis and intraabdominal abscess, caused by *Escherichia coli*, *Klebsiella* species, *Bacteroides* species including the *Bacteroides fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*), and *Clostridium* species.

^{††}Cefotan is a registered trademark of ICI Americas Inc.

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**These organisms, formerly considered subspecies of *B. fragilis*, have been given species status; however, because of similarities in their clinical significance and laboratory characteristics, they are often considered together as the "*B. fragilis* group."

[‡]The prescribing information for Cefotan lists no specific indication for infections caused by this organism.¹

gaps* in coverage?

All five of these *Bacteroides* are proven pathogens.

The evidence is conclusive.

fact: *B. fragilis* and *B. thetaiotaomicron* are the species of greatest clinical significance and are present in most intraabdominal infections.

fact: The prescribing information for Cefotan specifically states that most strains of *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron* are resistant.¹

fact: The absence of susceptibility of these *Bacteroides fragilis* species to Cefotan clearly shows that Cefotan does not have the same anaerobic spectrum as MEFOXIN.

Conclusion: Cefotan is not equal to MEFOXIN in activity^{††} against the *B. fragilis* group.

the
facts *still*
favor
Mefoxin[®] IV/IM
(Cefoxitin Sodium | MSD)

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use.

*i.e., *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron*

†† *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Prescribing information for Cefotan (Stuart), March 1986.

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For a Brief Summary of
Prescribing Information,
please see following page.

Mefoxin[®] $\frac{1}{4}$ IM (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTAXIME, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOTAXIME OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffe reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST II reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. **Local Reactions**—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and rarely, anaphylaxis. **Blood**—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

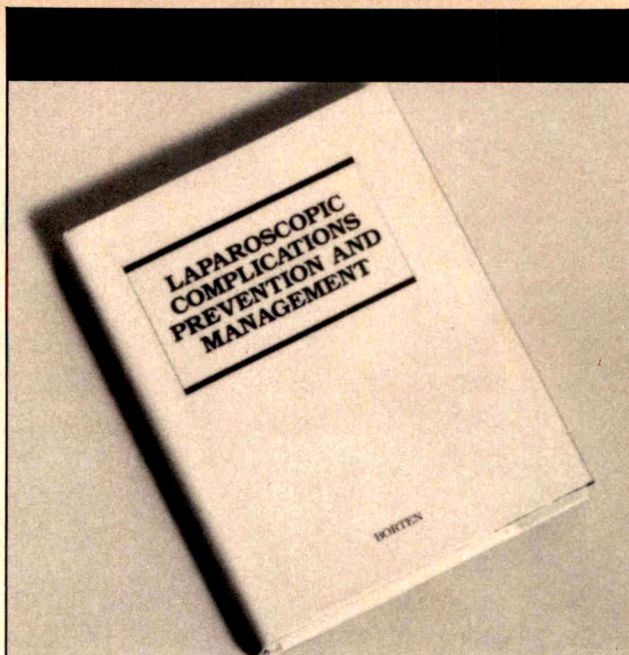
How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distans*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*
 || Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information. Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486

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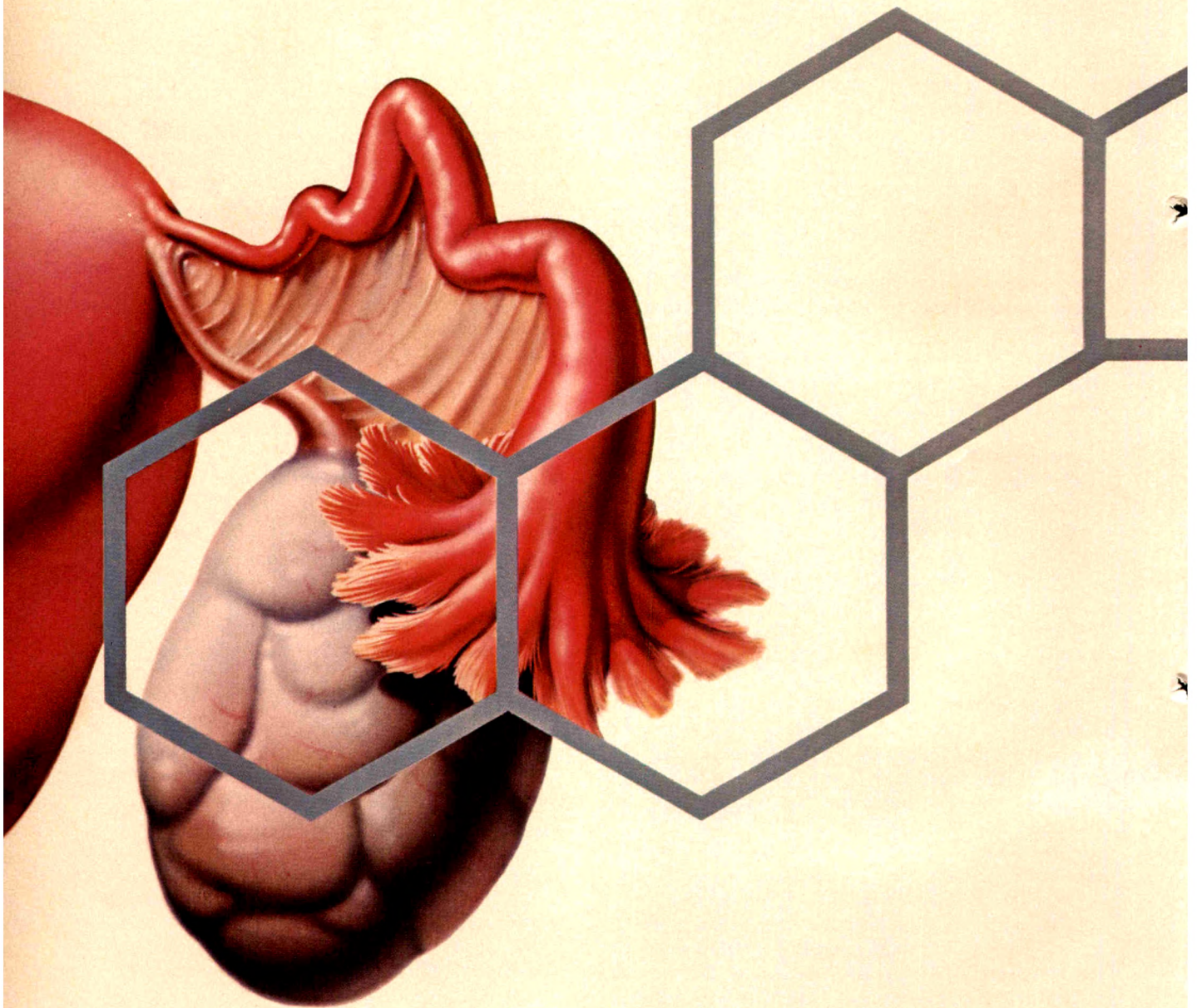
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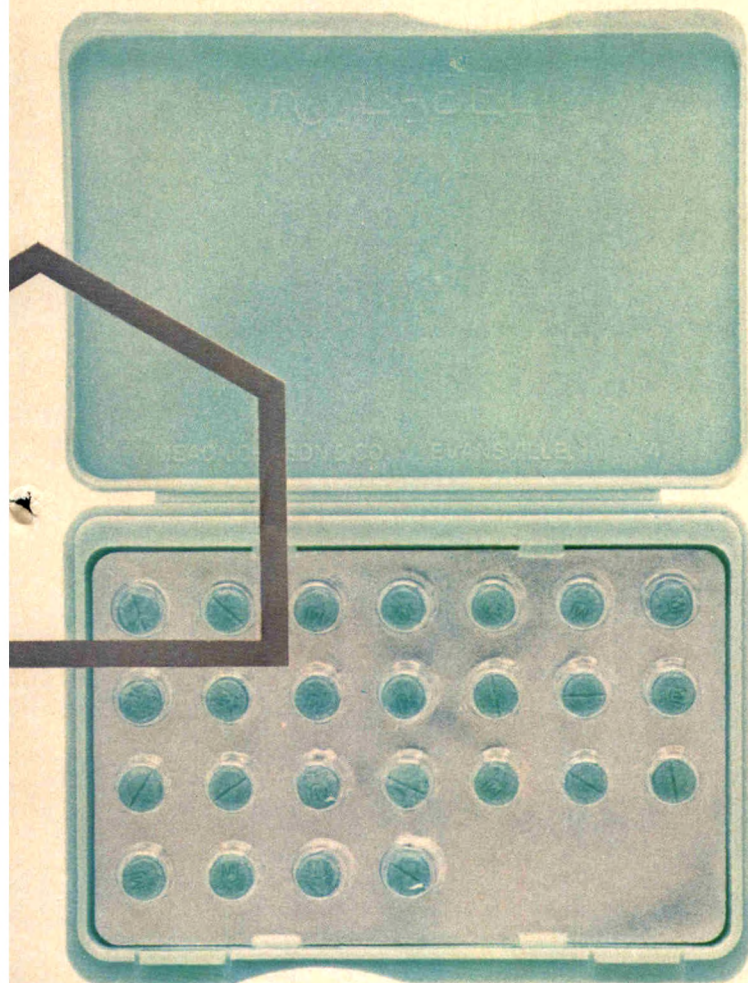
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EDITORIAL

Affiliation with the Society of Perinatal Obstetricians

The JOURNAL is pleased to announce that formal affiliation with the Society of Perinatal Obstetricians was consummated in 1986 with Dr. John Morrison, Director of Maternal-Fetal Medicine at the University of Mississippi Medical Center and President of this respected organization. This Society, composed of physicians who have special competence in maternal-fetal medicine, began in 1974 after the Certificate of Special Competence for Maternal and Fetal Medicine was inaugurated in 1973 by the American Board of Obstetrics and Gynecology. The last annual meeting of the Society had an attendance that exceeded five hundred registrants for formal and poster presentations. Dr. Frank Miller, Professor and Chairman of Obstetrics and Gynecology at the University of Arkansas, will function as the editorial liaison between the Society and Dr. Frederick P. Zuspan, Editor. In 1986, the October issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY has been designated for SPO publications.

The Editors and Publisher

Clinical Opinion

Update on prenatal steroid for prevention of respiratory distress

Report of a conference—September 26-28, 1985

Mary Ellen Avery, M.D., Glen Aylward, Ph.D., Robert Creasy, M.D., A. Brian Little, M.D.,
and Bitten Stripp, Ph.D.

Bethesda, Maryland

A workshop on approaches for enhancing pulmonary maturation and preventing neonatal respiratory distress syndrome in the 1980s was held in Washington, D. C., in September 1985, to consider the results of the Collaborative Study on Antenatal Steroid Therapy in the Prevention of Respiratory Distress Syndrome 1976-1983. Two papers describing major outcomes have been published,^{1,2} and a report including all findings of the trial was issued by the National Institutes of Health in August 1985.³ Obstetricians, pediatricians, psychologists, and biostatisticians were invited, including not only study participants but also independent critical observers. The discussion focused on an overview of the project, updates from the centers, follow-up, new basic research, and new approaches to prevent or manage respiratory distress syndrome.

In general, the results of prospective clinical trials can be viewed as either confirmatory of previous clinical experience, equivocal in failing to show either benefit, harm, or statistical significance, or provocative in revealing unexpected associations that may be of great significance.

Overall, the trial was confirmatory in showing a reduction in respiratory distress syndrome in the treated group. It was equivocal in showing no effect in the presence of multiple pregnancy and premature rupture of membranes and provocative in showing a statistically significant interaction between treatment effect and sex of the infant. A number of questions arose on further inspection of the data and were addressed.

Individual participants and their affiliations are listed at the end of the article.

The workshop was supported by the Division of Lung Diseases of the National Heart, Lung and Blood Institute, National Institutes of Health.

Reprint requests: A. Brian Little, M.D., Royal Victoria Hospital Women's Pavilion, 687 Pine Ave. West, Montreal, Quebec, Canada H3A 1A1.

A number of critics had questioned the enrollment criteria. At the five participating hospitals 7893 mothers in preterm labor were screened; 696 of them were randomized in the study. What happened to the others? Predetermined exclusion criteria were responsible for most of the exclusions (83.1%), including patients with anticipated delivery within 24 hours or after 7 days (accounting for more than 80% of the exclusions), those with intrauterine infection, those with a cervix dilated at least 5 cm at time of presentation, those who were Rh sensitized with amniotic fluid Δ OD for bilirubin in upper zone II or zone III (Liley), those who planned to give the baby for adoption, those on steroids, those with active peptic ulcer or tuberculosis, and those with lecithin/sphingomyelin ratio indicative of fetal pulmonary maturity. Cases in which the mother did not give consent (3.5%), the physician failed to enter patient (1.9%), requested the drug (0.2%) or with other contraindication (11.4%) were responsible for a much smaller proportion of exclusions.

The group questioned whether premature rupture of membranes in the absence of infection should preclude the use of dexamethasone. In this trial no benefit was seen when the drug was given to mothers with premature rupture of membranes. A reduction of respiratory distress syndrome incidence was observed in the steroid group (9.8%) versus the placebo group (12.6%), but this was not statistically significant. Some of the obstetricians present felt that a large leak with resulting oligohydramnios was a hazard with respect to subsequent fetal pulmonary hypoplasia after several weeks and that prenatal administration of dexamethasone with delivery after 48 hours was advisable. A small leak without oligohydramnios, however, may be another problem, and evidence in the literature for or against steroids is inconclusive.

The role of dexamethasone in multiple pregnancies

was discussed since no benefit was seen in the National Institutes of Health study. The question remains open, since none of the trials have had sufficient numbers to permit control for possible confounding variables such as sex, infection, and birth order. In Caspi et al.'s study,⁴ steroid was of benefit to the first-born, and in the collaborative study the second-born twin had more than twice the chance of having respiratory distress syndrome as the first-born (18/49 versus 8/50). It was suggested that the interaction of asphyxia and prematurity in promoting respiratory distress syndrome in the second-born may have been stronger than the putative steroid benefit. The question was raised whether the maternal dose was adequate in twin gestation, but a double-dose study failed to show greater benefit (Liggins GC, unpublished observations) and a few determinations of betamethasone levels in cord blood were not lower in twins (Liggins GC, unpublished observations) although dexamethasone levels in cord blood of twins were somewhat lower in the collaborative study. The question of use of prenatal dexamethasone in twins remains open, since none of the trials have had sufficient numbers to permit control for confounding factors such as sex and infection.

Why was dexamethasone used in this trial instead of betamethasone, which was used in the New Zealand trial⁵? Dexamethasone was chosen because it could be administered intramuscularly in a clear colorless solution, making the placebo preparation appear identical to dexamethasone (which is necessary in a blinded trial). Furthermore, laboratory studies had shown no difference in pulmonary receptor binding of the two steroids,^{6,7} and extensive pharmacokinetic data were available on dexamethasone from Merck Sharp & Dohme Co. Finally, labeled dexamethasone of high specific activity was already available, which was desirable for the development of the radioimmunoassay for subsequent determination of blood concentration. Smaller trials have shown benefit from dexamethasone.⁸

Is dexamethasone indicated when it is reasonably certain from ultrasonographic studies that the fetus is male? No statistically significant benefit was found in treated male infants of any race in the National Institutes of Health study. A review of other published trials provide conflicting results. About half of them report benefit to both sexes; about half show no benefit to males. The discussants agreed that the data are inconclusive and that the role of sex in response to antenatal dexamethasone deserves further evaluation, especially in the highest risk group—namely, those at risk for delivery before 28 weeks' gestation (<1 kg). One view expressed was, Why discriminate against the male fetus in light of evidence of benefit in some trials and absence of evidence of harm?

Is prenatal dexamethasone appropriate in gestations

that are <28 weeks? The collaborative study did not show benefit for that group. It was noted, however, that only 36 single births were <1 kg in birth weight, and 24 of them were from one center (Tennessee). The numbers were thought to be too few to be certain of no benefit. The discussants agreed that infants <28 weeks' gestation and <1 kg in birth weight were at greatest risk of respiratory distress syndrome and further study of combined hormone therapy (betamethasone and thyroid-releasing hormone) were indicated, especially in the low birth weight group. Current practice among the obstetricians present was to use corticosteroids in pregnancies of ≥ 27 weeks. Uncertainty was expressed about the wisdom of earlier administration.

Is a repeat dose of dexamethasone warranted after 7 days if delivery has not taken place? The argument for a repeat course is that the drug has been cleared by 1 week (as evidenced by blood levels) and that maternal adrenal function is no longer suppressed and any beneficial effects of an initial course of treatment are no longer apparent. The argument against this practice is that no clinical studies are reported to allay anxiety about possible cumulative adverse effects, such as suppression of fetal growth or brain development with longer exposure to prenatal corticosteroids. No conclusion was reached, other than that the arguments on each side of the question should be expressed.

The extrapulmonary effects of antenatal dexamethasone were discussed, and some of the participants believed that the benefits were sufficient to warrant advocating the use of this drug because of findings in this trial of significantly smaller incidence of necrotizing enterocolitis, bronchopulmonary dysplasia, and patent ductus arteriosus in the treated group. Others warned that the numbers were small and that the study had not been designed to evaluate these end points. All of the incidental findings, including a 4-day shorter average hospitalization in the treated group, could be the result of the reduction in incidence and severity in respiratory distress syndrome with the possible exception of necrotizing enterocolitis. It was agreed that these benefits were important, and recent data from animal studies supporting this belief were presented.

A developmental follow-up component of the study was planned to extend to 36 months. This was accomplished for 406 of a possible 739 infants. Ninety-three infants had died, but 240 were lost to follow-up, primarily because of geographic moves. The discussion group agreed that no differences in terms of cognitive, motor, or neurological function were found between steroid-exposed and placebo-administered infants. Cognitive functioning of both groups was adversely affected by lowered socioeconomic status and poorer social support. A general question was raised as

to the optimal duration of follow-up designed to evaluate the effects of a perinatal intervention or event. Short-term evaluation (9 months' corrected age) has the advantage of a better follow-up compliance (only 107 infants were lost at this time), and it allows better inference as to the relationship between the perinatal event and outcome. Longer follow-up enables more detailed assessment of higher-order cognitive function, growth, and later school achievements; however, the influence of a nonoptimal environment or intercurrent illness may overshadow any effects attributable to the original perinatal event. Given these considerations and the problems inherent in attempting to predict subsequent intellectual, motor, or neurological development from a single measurement early in life, a 3-year follow-up protocol seemed reasonable to the workshop participants. It should be emphasized that neither the type nor the magnitude of potential developmental changes that might occur as the result of corticosteroid treatment were known when the study was planned. Further, there were no data available to assist in calculating the number of infants needed for statistically valid follow-up studies. This discussion underscored the need to further evaluate these data and those of other studies in order to develop recommendations as to optimal duration and type of follow-up to be used in future clinical trials involving prenatal and perinatal interventions.

In a series of discussions of basic science findings and new pharmacologic approaches to enhance lung maturation, corticosteroids and thyroid hormones were shown to act independently and synergistically to stimulate phosphatidylcholine synthesis in whole animals and cultures of fetal human lung preparations. These effects appear to be receptor mediated, and the synergism is evidenced with a shorter time course than with either hormone alone. In addition, the effect of thyroid-releasing hormone, which stimulates triiodothyronine and prolactin plus cortisol, was examined in sheep in a series of experiments (Liggins GC, Schellenberg JC, Manzai M, Court DJ, unpublished observations). Triiodothyronine and cortisol were found to be synergistic in stimulating the secretion of alveolar-saturated phosphatidylcholine, being associated with increased fetal lung stability and distensibility only in the presence of increased prolactin. Thyroid-releasing hormone and cortisol were suggested to be of potential value in the clinical setting between 26 and 32 weeks of gestation, and an outline of a proposed clinical trial was reviewed.

Studies in the fetal rhesus monkey indicated that multiple small doses of glucocorticoid appeared to have an improved response over single dosage, with no differences being detectable between male and female fe-

tuses (Engle MJ, Kemnitz JW, Perelman R, Uno H, Farrell PM, unpublished observations). In preliminary studies in these animals in which there is marked motor cortex changes in contrast to the human fetus of similar age, there may be a dose-dependent, glucocorticoid-induced alteration in pyramidal cells and cytoarchitecture.

Accelerated intestinal maturation mediated by corticosteroids as evidenced by accelerated enzyme maturation, increased intestinal epithelial proliferation, and accelerated microvillus membrane maturation (decreased membrane fluidity) were discussed.⁹ The implications of these altered states on neonatal intestinal disease, particularly necrotizing enterocolitis, need further study. The complex response of patent ductus arteriosus in the lamb to different prostaglandins and prostaglandin inhibitors and oxygen was reviewed.¹⁰ From studies in preterm lambs it appears that the premature ductus is much more sensitive to the relaxing effects of prostaglandin E₂ than are near-term vessels, that circulating concentrations of prostaglandin E₂ are higher in preterm than in near-term animals, that cortisol significantly decreases the sensitivity of the ductus to prostaglandin E₂, and that triiodothyronine decreases the ability of the ductus to respond to vasoactive stimuli. Therefore, these data are consistent with the lower incidence of patent ductus arteriosus observed in infants prenatally exposed to corticosteroids.^{3, 10}

Investigations on artificial surfactant replacement therapy, whether cow-based or synthetic material, was briefly noted. Time of administration, lower age limit for therapy and some early results were discussed. The reported cloning of the apoprotein gene for surfactant was enthusiastically acclaimed.¹¹

The conclusion of this workshop was that antenatal dexamethasone to prevent respiratory distress syndrome was effective under the constraints that >24 hours before delivery was required to see an effect beneficial to female infants of >30 weeks' gestation. Many special circumstances were discussed with opinions expressed about current practices by the participants in the study. It was agreed no therapeutic trial can provide all the answers; where the data were equivocal or numbers insufficient, it was apparent more research is needed. These included circumstances with premature rupture of membranes, multiple pregnancies, male sex of the fetus, pregnancies under 30 weeks' gestation, and the question of a repeat dose of steroid after 7 days.

The Collaborative Study was one facet of all the steps toward the elucidation of the resolution of a major hazard of premature delivery, which will only be obviated by the resolution of premature labor itself.

Participants in the workshop included the following:

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Pregnancy events and brain damage

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A national standard of perinatal care has been established for the early detection and appropriate management of the fetus-neonate who is at risk for distress, brain damage, or death. While perinatal and infant mortality has significantly decreased during the past 10 years, a similar reduction in conditions of severe handicap has not been observed. Undetected antenatal umbilical cord compression is suggested as a possible cause of unexplained brain damage. Basic animal experimentation and clinical experience is reviewed to support the hypothesis. (AM J OBSTET GYNECOL 1986;155:6-9.)

Key words: Pregnancy events, brain damage, cord compression

Pregnancy events leading to brain damage are poorly understood. That was the conclusion of a recent review sponsored by the National Institutes of Health¹ and is the opinion shared by most clinicians and scientists who have conducted clinical or basic science research in this area. Why is it that a problem so costly to the family and society in terms of emotional and financial expenditures has eluded understanding? Advances in perinatal care have dramatically increased perinatal survival but the quality of life as measured by long-term morbidity has not been significantly affected. There is no firm evidence that any significant reduction in handicapping conditions (cerebral palsy, mental retardation, and epilepsy) has paralleled the decrease in perinatal and infant mortality that has occurred during the past 20 years. The reasons for this are not clear.

The introduction of techniques for intrapartum fetal surveillance has resulted in earlier detection and management of fetal stress-distress conditions. Perinatal hypoxia-asphyxia is identifiable by electronic and biochemical monitoring in both obstetric and pediatric intensive care units. Only acute obstetric emergencies such as an abruptio placentae or a cord prolapse may present without prior warning and appropriate medical preparation. It is now known from long-term, prospective follow-up studies that only a very small percentage of "birth asphyxiated" newborn infants, defined as infants with Apgar scores of ≤ 6 at 5 minutes, develop handicapping conditions.^{2,3} It has been estimated that no more than 10% to 20% of handicapped infants have a documented history of "birth asphyxia." Three quarters of the handicapped population reveal

no evidence of perinatal hypoxia on careful review of pregnancy and newborn records.

Increased survival of the very low birth weight newborn infant (<1500 gm) could potentially contribute a larger group of infants at risk for handicap. However, only one third of the handicapped have a history of low birth weight while two thirds have birth weights >2500 gm. It can be concluded that while perinatal hypoxia-asphyxia can result in brain damage in both the term and low birth weight infant it cannot be etiologically related in the majority of cases of handicap.

Major known causes of handicapping conditions in addition to perinatal hypoxia-asphyxia involve mutant genes, chromosomal anomalies, prenatal environmental teratogens, perinatal infections, inborn errors of metabolism, and other less frequent factors. One of these factors will be identified in approximately half of patients with known handicaps. Genetic, chromosomal, and teratogenic factors will be discovered more frequently in association with mental retardation than with cerebral palsy. Unless associated with clinical stigmata genetic chromosomal or teratogenic abnormalities would not be suspected or investigated at birth or during the neonatal period. Rapidly advancing technology in the genetic field may clarify these issues in the near future.

At least half, if not more, of all brain-damaged infants and children have no scientifically or clinically based explanation for their problem. The usual history reveals a child who is born following an uncomplicated pregnancy, labor, and delivery, with Apgar scores of 8 and 10 and a normal neonatal course, who later shows developmental delay in motor and/or mental functioning and is eventually diagnosed as handicapped. What is the cause of such an insult to the brain and when did the injury occur? Is it possible that the injury occurred at some point during the antenatal period before labor and delivery? Is it possible that this injury resulted from

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a spontaneous cord complication that led to a transient fetal hypoxia-asphyxia episode not severe enough to result in death but significant enough to irreversibly damage critical areas of the developing brain? In view of the fact that many handicapping conditions occur in the midst of a normal uncomplicated obstetric and neonatal history such a hypothesis must be considered and evaluated. Actually a significant amount of evidence from animal experimentation and human clinical experience exists to support this hypothesis.

Animal experimentation to support cord hypothesis

The fetal brain has been investigated by many disciplines with the use of many different animal preparations. Initial experimentation was designed to describe normal development and ontogenetic patterns of anatomy, biochemistry, and function. The reports by Windle⁴ were some of the earliest to correlate neuropathology with environmental abnormalities. The original studies were conducted in monkey fetuses that were subject to cord clamping at birth for various intervals prior to the initiation of respiration. These studies of "asphyxia neonatorum" showed neuropathologic lesions primarily in the brainstem and hypothalamus. This perinatal insult did not reveal pathologic evidence of significant cerebral white or gray matter leukomalacia or hemorrhage that is most often found at autopsy of infants with documented perinatal hypoxia or of children that died with known severely handicapping conditions. Further investigation by Myers⁵ in the monkey and by our own group⁶ in the acute and chronic sheep preparation have revealed white matter lesions that are similar to the leukomalacia seen in humans. These lesions were noted when the animal preparation was subjected to prolonged acute or chronic hypoxia that resulted in a significant metabolic acidosis and accumulation of lactate. Analyses of our data indicated that before pathologic evidence of brain damage clear patterns of cardiovascular instability and electrocorticographic abnormality could be recognized. Ominous fetal heart rate-uterine contraction deceleration patterns could be identified before evidence of interference with brain function and structure. These studies in the fetal lamb along with those conducted by Myers in the monkey served eventually as the basic animal data to support the pioneering work of Hon⁷ in human fetal heart rate-uterine contraction monitoring. Unfortunately human fetal electroencephalography explored by Rosen et al.⁸ and by us⁹ has to date been limited by technologic problems of overcoming a low signal-to-noise ratio.

As much of our original animal experimentation was done with acute or subacute preparations, an attempt

to repeat the investigation in the chronic preparation was begun by Clapp et al.¹⁰ at the University of Vermont. The chronically instrumented fetal sheep preparation with a known growth-retarded fetus following microembolization of the uterine circulation was used for study. The growth-retarded fetus was then subjected to an acute reduction in uterine blood flow by an epinephrine infusion. The fetal brain was perfused and fixed in situ and sent along with similarly perfused control brains for neuropathologic examination. The neuropathologist was unaware of the experimental conditions. Reports from the neuropathologist revealed the expected white matter lesions. However, after these cavitory lesions were dated as to length of injury, it was clear that the injury had been sustained many days before the acute experimental stress. The lesions also were found in some control animals. Careful analysis of intermittently determined metabolic and acid-base data during several days or weeks before the animals were put to death failed to show any significant deviation from accepted normal values for the chronic preparation. Careful examination of heart rate and umbilical blood flow recordings that were obtained during several days revealed that in each of the experimental and control animals showing white matter lesions a pattern of vascular instability consistent with cord compression was evident. Electrocorticographic abnormalities could be detected during the episodes of vascular instability.

With this information available it was decided to prospectively evaluate the effect of intermittent cord compression on brain function (electrocorticogram) and pathologic findings.¹¹ A chronically instrumented fetal preparation was used that had an inflatable cuff placed around the umbilical cord. Partial, intermittent cord compression was performed for arbitrarily selected periods and frequencies. The animal was put to death and brain fixation was done 3 days after cord compression. White matter lesions were found in all animals studied and interestingly in two control animals who showed evidence of spontaneous cord compression.

What has been learned from these earlier and more recent animal experiments is that a selective vulnerability of the developing white matter exists. It has been shown that the white matter is vulnerable to hypoxia with significant acidosis and lactic acidemia as well as intermittent cord compression without metabolic acidosis. The cardiovascular physiologic mechanism to explain the documented pathologic findings is unknown at the present time. It most likely involves a redistribution of cerebral blood flow under these conditions with hypoxic cellular damage and necrosis occurring when areas of susceptible white matter are hypoper-

fused. Volpe et al.¹² have suggested this pathophysiology from exciting observations of human newborn infants with positron emission tomography. Further experimentation is required to support this hypothesis.

Clinical evidence to support the cord compression theory

As the experiments referred to above indicate that human fetal heart rate—uterine contraction and biochemical monitoring detect fetal hypoxia-asphyxia before neuropathologic damage, widespread application of such techniques should result in early recognition, management, and avoidance of such complications. During the intrapartum period both uteroplacental insufficiency of any etiology and cord compression can be diagnosed and managed appropriately. With a national standard of care that involves routine surveillance of fetal condition during labor, fetal mortality and morbidity during this period should be limited to acute, unpredictable obstetric emergencies.

During the antepartum period, however, diagnostic evaluation of fetal condition concerns itself primarily with the detection of fetal stress by nonstress test—contraction stress test monitoring. A nonreactive nonstress test and/or positive contraction stress test involving delayed decelerations elicits concern and clinical urgency. Much less attention is paid to the appearance of an isolated or nonpersistent deceleration with a rapid onset and recovery that is seen during the performance of a nonstress test—contraction stress test. While cord compression is suspected the clinical situation is not considered ominous. The suspicious test is generally repeated within 24 hours and if no decelerations are noted the patient is discharged. If the decelerations persist and the fetus is mature, labor may be induced and management based on the appearance and severity of the variable decelerations.¹³

Transient decelerations in fetal heart rate or prolonged bradycardias found during antepartum visits before fetal maturity present a major clinical dilemma. Every obstetrician has noted on occasion a bradycardia of <100 bpm, turned the patient, made pleasant conversation, and then on reauscultation some minutes later recorded a perfectly normal heart rate. Patient inquiry of the problem elicits an explanation that usually involves cord compression but reassurance of a healthy baby. How frequent an episode of this type occurs in the 24-hour day of the pregnant woman is unknown. It is likely increased with a short or long cord, cord entanglements, knots in the cord, or significant oligohydramnios.¹⁴ Some abnormality of the cord will be found in 30% of all deliveries.

Until the introduction of ultrasonographic evaluation of the fetus it would not have been possible to determine potential antepartum cord complications.

Recent reports indicate that the nuchal cord can be recognized by careful ultrasonographic evaluation.¹⁵ Ultrasonographic evaluation of the fetus should routinely include more comments regarding the cord than presently provided. This is of particular importance when ultrasonographic evaluation is conducted for episodes of spontaneous fetal bradycardia or for a suspicious nonstress test—contraction stress test with variable decelerations. Reports of noninvasive measurement of umbilical blood flow by pulsed Doppler techniques further enhance the opportunity to study this question.¹⁶ Pulsed Doppler flow studies give every indication that the effect of cord compression on umbilical blood flow can be determined.

Both basic science and clinical research protocols must be developed to study this fertile area of investigation. Long-term prospective controlled studies of motor and mental development of newborn infants selected because of antenatal cord complications are required to prove the hypothesis. Management and treatment options for umbilical cord complications will follow an understanding of the basic pathophysiologic mechanisms.

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The indirectly obtained fetal heart rate: Comparison of first- and second-generation electronic fetal monitors

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Fetal heart rate variability has become an essential component in evaluation of the fetus during continuous electronic fetal monitoring. Because of technical deficiencies of monitoring equipment, fetal heart rate variability may not always be available, such as during indirect monitoring of the fetus. In lieu of integration, a mathematical procedure called autocorrelation has, with the use of microprocessors, been incorporated into the fetal monitor, creating the "second generation" of electronic fetal monitoring. This study examined the accuracy of the first- and second-generation monitors by comparing simultaneously obtained indirect and direct real-time fetal heart rate data. The second-generation monitor produced an indirectly obtained fetal heart rate that was more accurate when compared with the directly obtained data than that with the first-generation fetal monitor. In addition, the variation in data from the first-generation monitor was substantially higher than that with the second-generation monitor. It is concluded that the fetal heart rate tracing from the second-generation monitor is more accurate when compared with the fetal electrocardiogram and may facilitate a truer interpretation of fetal heart rate variability than that obtained with the first-generation monitor. (AM J OBSTET GYNECOL 1986;155:10-14.)

Key words: Fetal monitoring, variability, autocorrelation

Fetal heart rate (FHR) variability has become an essential component in evaluation of the fetus during continuous electronic fetal monitoring. Interpretation of FHR variability has been shown to aid in the clinical management of labor¹⁻³ and to predict neonatal outcome.⁴⁻⁶ However, because of technical deficiencies of fetal monitoring equipment, FHR variability may not be available in some circumstances. Specifically, assessment of the fetal heart rate and rhythm by the indirect (external) method has not provided accurate information for interpretation of FHR variability because of imprecise measurement of fetal cardiac interval distances.⁷ When FHR variability interpretation is required, a fetal scalp electrode must be applied, often-times necessitating artificial rupture of amniotic membranes, which may cause complications when the procedure is difficult or contraindicated. Past attempts to reliably obtain FHR variability indirectly with phon-

ocardiography and abdominal electrocardiogram have not been successful because of other technical problems⁸ (Boehm FH, Salyer S. unpublished data).

Recently, a principle called autocorrelation has been incorporated into the electronic fetal monitor to improve the fetal heart rate signal and accuracy of FHR variability interpretation when external appliances are used. Because computer technology enables real-time computation of autocorrelation we have previously named this new electronic fetal monitoring "second generation."⁹

Both first- and second-generation fetal monitors use Doppler ultrasound to detect fetal heart motion. The ultrasound transducer emits an ultrasound signal and receives the reflected signal, which has been Doppler shifted by heart movement. The received signal is converted to an electrical waveform by the transducer. Although detection is similar between first- and second-generation fetal monitors, the processes applied to convert signal to FHR are different. First-generation monitors calculate heart rate by integration, and second-generation monitors by autocorrelation. Electronic integration sums the area under the previously received waveform until some predetermined voltage level is achieved. A counter is set in action until the next heart-

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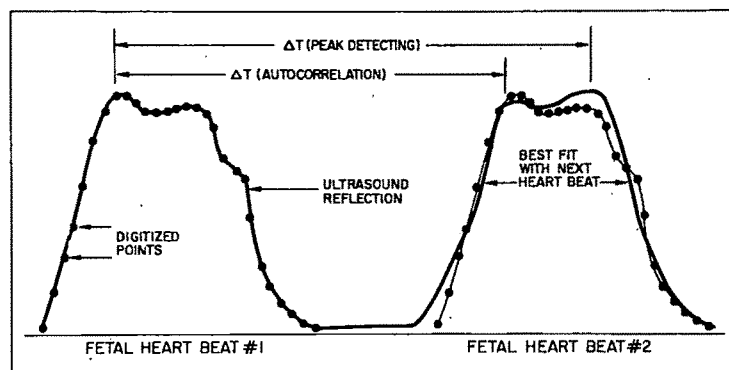


Fig. 1. Waveforms of two ultrasound cardiac signals compare integration (peak-to-peak) of the first-generation electronic fetal monitor with autocorrelation of the second-generation electronic fetal monitor. (Used by permission, Hewlett-Packard, Palo Alto, California.)

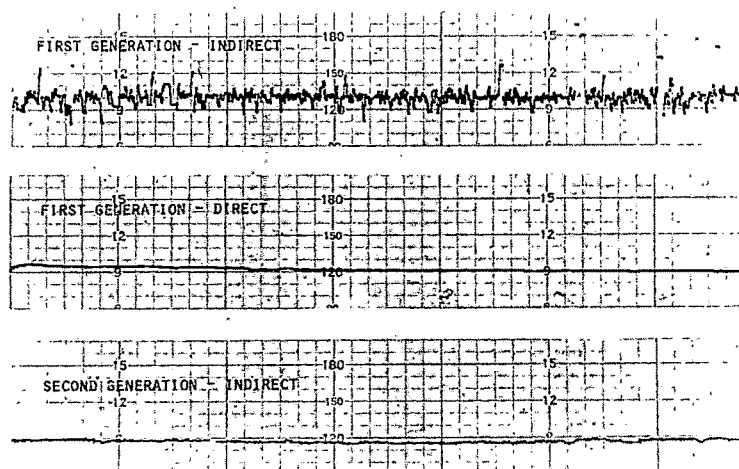


Fig. 2. Simultaneous FHR tracings compare direct and indirect methods of first-generation equipment with indirect method of second-generation equipment.

beat is received. As the second beat is received the count is stored and the counter is reset to zero; the stored count is converted and displayed as beats per minute on a numerical display and a point on the chart recorder. The cycle is then repeated with each successive beat. The introduction of high-speed microprocessor integrated circuits has made autocorrelation monitoring possible. Autocorrelation is a mathematically intense procedure that builds a pattern of the received Doppler waveform. Each pattern is described as points along the waveform (Fig. 1). When a new heartbeat waveform arrives from the Doppler section, the microprocessor compares it with the pattern and updates the signals going to the numerical display and chart recorder. In addition, new points are collected to update the internal pattern and the oldest points are discarded.

In an early study of 23 patients,⁹ first- and second-generation indirect electronic fetal monitor data were visually compared with simultaneously obtained data from fetal scalp electrodes. A strong visual correlation

was noted between indirectly obtained second-generation FHR data and directly obtained FHR data (Fig. 2). Lawson et al.¹⁰ reported a comparison of second-generation indirect fetal monitoring to direct fetal electrocardiogram detection in a group of 10 patients. They concluded that reduction in signal loss was achieved with minimal sacrifice in accuracy. Most recently, Divon et al.¹¹ visually compared first- and second-generation indirect fetal monitoring to direct fetal electrocardiogram detection in two patients. They demonstrated superior performance of the second-generation monitor in the presence of external noise interference. This report proposes to compare indirect first- and second-generation FHR data with simultaneously obtained fetal electrocardiogram data.

Material and methods

For the purpose of this study, two Corometrics (Wallingford, Connecticut) fetal monitors were chosen as representative of first-generation equipment, and a

Hewlett-Packard (Palo Alto, California) fetal monitor as representative of the second generation. The Corometrics Model 110 was selected for ultrasound capabilities and the Corometrics Model 101B was chosen as the electrocardiogram monitor. A Hewlett-Packard Model 8040A was used for comparison. This instrument has simultaneous electrocardiogram and ultrasound capabilities.

The collection of patient data was carried out in two consecutive phases. In phase one, the two Corometrics fetal monitors were interfaced to an Apple II computer (Cupertino, California) to collect patient data. In phase two, the Corometrics monitors were replaced by the Hewlett-Packard fetal monitor and interfaced to the same microcomputer for data collection. In each phase of the test, both the electrocardiogram and ultrasound units were simultaneously monitoring the fetus. The heart rate signals from the electrocardiogram and ultrasound appliances were sampled in immediate succession, once each second by the microcomputer, and then stored on a floppy disk. The elapsed time between the electrocardiogram and ultrasound data samples was approximately 90 msec.

Heart rate signals from both Corometrics monitors are analogue voltages that are proportional to the measured heart rate.^{12,15} A single analogue to digital converter handled the conversion of both the electrocardiogram and ultrasound voltages to binary signals. This technique effectively offsets any errors introduced during the analogue to digital conversion process, since the information of primary interest was the difference between each electrocardiogram-ultrasound data pair. The fetus was monitored by both an electrocardiogram scalp electrode and an ultrasound device for simultaneous monitoring. The output analogue voltages representing heart rate data flow from both Corometrics monitors to a common analogue to digital converter. There is an electronic switch controlled by the microcomputer, which connects one monitor at a time to the analogue to digital converter input. The Corometrics analogue signals are available continuously; thus it is not necessary to interrogate the monitors. The binary coded output from the analogue to digital converter is then routed directly to the microcomputer. These binary numbers are then decoded into their decimal equivalents by the microcomputer before storage on the floppy disk.

The data output channel of the Hewlett-Packard monitor is a 20 mA current loop, bidirectional, serial port. Through this port, the monitor receives commands and transmits data to external devices. Because the microcomputer did not support a current loop input, a current loop to RS-232 converter was installed between it and the monitor. The necessary Hewlett-

Packard command sequences are well documented in the manufacturer's manual and will not be repeated here.^{14,15}

Data transmitted from the Hewlett-Packard electrocardiogram and ultrasound monitors are output in two binary chunks (bytes). The first contains the least significant binary digits and the next byte contains the most significant digits plus other information concerning the status of the monitor. To reassemble the heart rate information, the microcomputer must remove the status flags and then decode the two binary bytes into a single decimal number. This decimal value is the measured heart rate multiplied by a factor of four. After division by four the actual heart rate data are ready to store on a floppy disk for data reduction at a later time.

Ten randomly selected subjects were studied in phase one. All subjects were in labor and had ruptured amniotic membranes and a fetal scalp electrode in place. The scalp electrode was connected to the Corometrics 101B by a leg plate appliance. The Doppler ultrasound was placed on the maternal abdomen, carefully positioned for maximum signal, and connected to the Corometrics Model 110. During each data collection sequence in phase one, 1000 data points were collected from each subject. The sequence was not repeated. Fifteen randomly selected subjects were studied in phase two. As in phase one, all subjects in phase two were in labor and had ruptured amniotic membranes and a fetal scalp electrode in place. The scalp electrode was connected to the Hewlett-Packard 8040A by a leg plate appliance. The Doppler ultrasound device was placed on the maternal abdomen, carefully positioned for maximum signal, and connected to the same Hewlett-Packard fetal monitor. During each data collection sequence, 255 data points were collected. This sequence was repeated three times in immediate succession on each subject. During both phases, the monitor was observed for a time before the start of data collection to ensure that signal dropout caused by initial transducer placement would be minimal. Subjects were instructed to remain in a stationary position during data collection and the ultrasound transducer was not repositioned during that time.

The data were originally obtained in real time on 5¼-inch diskettes with the Apple II microcomputer. For purposes of statistical analysis the data were transferred from the Apple microcomputer to the DEC VAX 11/780 computer (Maynard, Massachusetts) with the use of communication software. SPSSX (1) (Statistical Package for the Social Sciences) was used to determine the frequency distribution of the absolute difference between the electrocardiogram and ultrasound FHR data for both phases of the study. The purpose of comparing the distribution of differences between monitors

Table I. Means and variances in beats per minute of the first- and second-generation fetal monitors

	Phase one (first generation)	Phase two (second generation)	p Value
Mean of difference	10.9	2.5	$<10^{-4}$
Variance of difference	628.7	51.8	$<10^{-4}$

was not only to determine if the average difference in beats per minute between the monitors was different but also to see if one monitor was inherently more variable than the other. The F test was used to compare the variances between the two monitors, and comparison of the differences in the means was done by means of the *t* test adjusted for unequal variance in the two groups. In addition, a test of the difference in the frequency distributions was done with the Kolmogorov-Smirnov tests.

Results

There was no statistical difference between patient's gestational age or cervical dilatation at the time of FHR data collection between phase one and phase two.

The means and variances for the first- and second-generation fetal monitors are shown in Table I. The average of the absolute difference between electrocardiogram and ultrasound data pairs obtained with the use of the first-generation monitor was 10.9 and that with the second-generation monitor was 2.5 ($p < 10^{-4}$). In addition, the variation in data from the Corometrics monitor was substantially higher than that with the Hewlett-Packard monitor ($p < 10^{-4}$). The absolute difference in beats per minute between electrocardiogram and ultrasound data pairs is shown as the cumulative frequency in Table II for the first- and second-generation monitors. The cumulative frequency distributions are significantly different (Kolmogorov-Smirnov $p < 10^{-4}$).

Comment

The inability to interpret FHR variability by indirect electronic fetal monitoring has oftentimes hindered the physician's ability to distinguish fetal stress from distress. Frequently, criteria for determination of fetal health have been dependent on the status of the amniotic membranes. When membranes are ruptured and placement of the fetal scalp electrode is possible, FHR variability may be the primary parameter for fetal assessment. When rupture of the membranes and direct fetal monitoring are not possible other FHR baseline characteristics and periodic patterns form the criteria for fetal assessment. The results of this study indicate

Table II. Absolute difference in beats per minute between electrocardiogram and ultrasound data pairs for first- and second-generation fetal monitors

Beats per minute	Phase one (first generation)		Phase two (second generation)	
	Cumulative frequency	%	Cumulative frequency	%
0	972	9.7	641	6.1
1	2,834	28.3	4,843	46.3
2	4,489	44.9	7,406	70.8
3	5,826	58.3	8,643	82.7
4	6,770	67.7	9,276	88.7
5	7,397	74.0	9,644	92.2
Total	10,000	100.0	10,455	100.0

$p < 0.001$ (Kolmogorov-Smirnov).

that the newer second-generation electronic fetal monitor with autocorrelation capability produces an FHR baseline significantly more comparable to directly obtained data than that with the first-generation monitor.

The second-generation fetal monitor provided an FHR signal that was within an average of 2.5 bpm of the fetal heart rate detected by the direct fetal electrocardiogram. In comparison, the first-generation fetal monitor provided an FHR signal that was within an average of 10.9 bpm of the directly obtained fetal electrocardiogram. As noted in Table II, the second-generation fetal monitor was within 3 bpm of the directly obtained fetal electrocardiogram 82.7%, as compared with 58.3% of the time for the first-generation fetal monitor. FHR variability is an important parameter in assessment of fetal health when the FHR is electronically monitored. The more reliable data obtained by the newer second-generation monitor will promote more precise fetal evaluation when the fetus is externally monitored. These findings lead to significant implications for management when delivery of the high-risk patient with an unripe cervix is indicated.

Most of the major fetal monitor manufacturers are producing equipment with autocorrelation processing of the FHR. Caution should be exercised in the generalization of these study results to all brands of fetal monitors. Comparison studies to define the variation in beat-to-beat differences between directly and indirectly obtained FHR data will be necessary for each new fetal monitor presenting a form of autocorrelation.

In summary, the present study supports the use of the Hewlett-Packard 8040A fetal monitor to indirectly obtain a more accurate FHR tracing. The interpretation of FHR variability from an external tracing appears to be more reliable when this second-generation fetal monitor is used than when the first-generation monitors are used.

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The "vanishing twin": Ultrasonographic assessment of fetal disappearance in the first trimester

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Review of the sonographic findings of 1000 pregnancies with viable gestations in the first trimester revealed a minimum incidence of twinning of 3.29%. Of these, 21.2% demonstrated the "vanishing twin" phenomenon, often with associated bleeding, but with a good prognosis for the remaining fetus. Ovulation induction did not appear to alter these data. The sonographic criteria for "vanishing twin" are discussed. The incidence of multiple gestation is 3.29% to 5.39%, higher than previously believed. (*AM J OBSTET GYNECOL* 1986;155:14-9.)

Key words: "Vanishing twin," early fetal loss, multiple gestation

Documentation of early human fetal loss in multiple gestation was difficult before the use of ultrasound. A previous paper reviewed nine separate reports that documented the phenomenon of the "vanishing twin."¹ These studies reported (1) that serial ultrasound examinations could demonstrate the "disappearance" of at least one of two gestational rings and (2) that the number of twins observed at delivery was markedly less

than the number of twin pregnancies identified sonographically in the first trimester. Since that review, other investigators have confirmed the occurrence of the "vanishing twin."²⁻⁴

Disappearance rates of fetuses in the reports cited range from 0% to 78%. This wide range is related to several factors, including differences in study technique, sample size, and incorrect interpretation of either technical ultrasound artifacts or physiologic conditions associated with pregnancy which can mimic the presence of an additional gestational sac. An accurate assessment of the rate of disappearance of human fetuses is a prerequisite to establishing the true conceptual rate of multiple gestations in humans. This information can affect the management of individual pregnancies.

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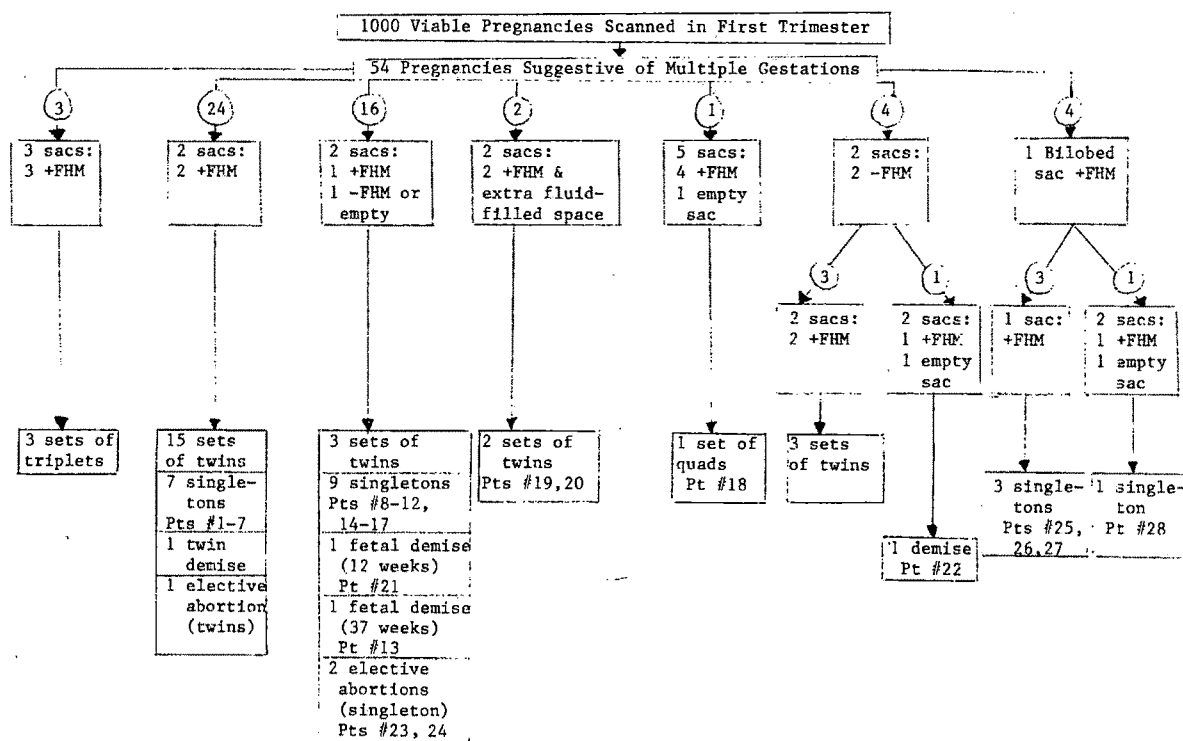


Fig. 1. Patient population, initial scan information, and pregnancy outcomes. Patient numbers correspond to assigned numbers in Tables I, II, and III. +FHM = Presence of fetal heart motion. -FHM = Absence of fetal heart motion; implies fetal pole or parts seen. Empty sac = No evidence of fetal pole or parts seen.

The present study was undertaken in an attempt to establish an accurate rate of disappearance of human fetuses based on ultrasound examinations in the first trimester.

Material and methods

One thousand pregnancies were evaluated by first-trimester ultrasound examinations at Pennsylvania Hospital, Philadelphia, Pennsylvania, between 1982 and 1984. In each pregnancy, fetal viability was documented ultrasonographically by viewing fetal heart motion. The ultrasound examinations were performed by several different examiners with an experienced ultrasonologist supervising. Multiple gestation was suggested in 54 pregnancies (Fig. 1).

In describing the sonographic data obtained in this study, it should be emphasized that the use of the phrase "empty sac" implies no sonographic evidence of fetal parts or a fetal pole; "absent fetal heart motion" implies the inability to visualize fetal heart activity and the ability to see ultrasonically a fetal pole, fetal parts, or internal echoes.

Results

Of 54 patients with suspected multiple pregnancies, 26 went on to deliver 26 sets of multiples and 28 were found to have a vanishing fetus (Tables I to III). Three distinct groups were defined.

Pregnancies in which fetal heart motion was documented in two gestational sacs and subsequent scan revealed one viable fetus constituted the group with documented diagnoses (patients 1 through 7, Table I). The group with suspected diagnoses was composed of 17 patients whose initial ultrasound evaluation revealed one or more viable gestations and an additional sac that was empty or abnormal; the final number of viable fetuses was one less than the number of sacs visualized sonographically (patients 8 through 24, Table II). The group with doubtful diagnoses was represented by four pregnancies in which a viable fetus was found to be present within a bilobed gestational sac, and the possibility of a twin gestation could not be excluded (patients 25 through 27 in Table III).

Overall, there were one set of quadruplets, three sets of triplets, 25 sets of twins, and 25 singleton gestations. Fig. 1 illustrates the pregnancy outcomes.

Vanishing fetuses. All seven patients in the group with documented diagnoses had viable twins on ultrasound evaluation and subsequent scan revealed a singleton gestation (Table I). Five patients were delivered of term singletons, one pregnancy was terminated for genetic reasons, and one pregnancy was complicated by bleeding and subsequent fetal death at 13 weeks' gestation.

In the group with suspected diagnoses the ultrasound findings of 17 pregnancies revealed one or more

Table I. Vanishing twins—group with documented diagnoses

Patient	Indication for scan	Gestational age at first scan (wk)	Findings	Gestational age at next scan (wk)	Findings	Pregnancy information	Outcome
1	GD	6	2 empty sacs	8	2 sacs, +FHM	Artificial ovulation; spotting at 6 weeks	Term singleton
2	GD	7	2 sacs, +FHM	9	1 sac, +FHM 1 sac, -FHM	—	Term singleton
3	GD	7½	2 sacs, +FHM	9½	1 sac, +FHM	Artificial ovulation	Term singleton
4	GD	8	2 sacs, +FHM	16½	1 viable fetus	Amniocentesis at 16½ weeks	Trisomy 21; termination
5	GD	8	2 sacs, +FHM	10	1 sac, +FHM	Spotting at 9 weeks	Term singleton
6	GD	11	2 sacs, +FHM	13½	1 sac, +FHM	—	Term singleton
7	GD	8	2 sacs, +FHM	13	1 small sac, -FHM	Bleeding at 13 weeks	Singleton death

GD = Gestational dating. +FHM = Presence of fetal heart motion. -FHM = Absence of fetal heart motion; implies fetal pole or parts seen. Empty sac = no evidence of fetal pole or parts seen. R/O = Rule out.

viable gestations progressing alongside an additional empty or abnormal sac (Table II). The scans of two pregnancies showed three gestational sacs, only two of which contained fetal heart activity; these pregnancies resulted in the delivery of term twins. Another patient had an early scan that revealed five sacs, four of which contained viable fetuses, and immature quadruplets were delivered at 22 weeks' gestation. Among the remaining pregnancies in this group, nine patients were delivered of term singletons, three fetal deaths occurred (two in the first trimester and one at term), and two pregnancies were electively terminated.

The ultrasound findings of the four pregnancies in the group with doubtful diagnoses confirmed a viable fetus within a bilobed sac (Table III). On subsequent scans, viable singletons were identified in all pregnancies, and in one case an additional empty sac was seen. These patients all were delivered of singletons.

The overall disappearance rate for one of twin fetuses obtained in this series is 21.2%, considering only those patients in the group with documented diagnoses. The disappearance rate increases to 48% and 51.8% if the respective groups of suspected and doubtful diagnoses are considered.

Use of ovulation induction. Of the 54 patients suspected of having ultrasound evidence for an early multiple gestation, 19 pregnancies (35.2%) were conceived with ovarian stimulation. Ten patients were delivered of multiples (three sets of triplets and seven sets of twins), and nine patients (16.7%) had pregnancies that involved a vanishing fetus.

First-trimester bleeding. First-trimester bleeding occurred in a total of 10 patients (18.5%). Seven of these patients were among the 28 pregnancies with a vanished fetus (25%), and two losses occurred in the first trimester (28.6%). In comparison, only 68 of 946 patients reported bleeding in the first trimester (7.2%),

and among these cases, 11 first-trimester losses occurred (16.2%).

Incidence of multiple gestation. The incidence of multiple gestation derived from the present data is 3.29%, which includes only those patients with documented vanishing fetuses. Inclusion of patients in the groups with suspected and doubtful diagnoses increases the incidence of multiple conception to 4.99% and 5.39%, respectively.

Comment

The true rate of postconception loss in multiple gestations is not known. Reports of vanishing fetuses based on serial ultrasound examinations suggest that the rate of conception of multiple pregnancy in humans is significantly higher than previously appreciated.¹⁻⁴ A prior publication included a literature review of nine separate studies that reported early fetal loss in multiple gestation with use of sonography.¹ These studies noted the disappearance of one or more gestational sacs on serial ultrasound scans and the fact that considerably fewer twins were observed at delivery than had been identified by ultrasound in the first trimester. Varying disappearance rates were cited, ranging from 0% to 78%.¹ The present study examined 1000 pregnancies with first-trimester ultrasonography in attempting to accurately estimate the disappearance rate of human fetuses in multiple gestation.

The disappearance of a fetus may be explained by physiologic processes or by false interpretation of either normal physiologic conditions or artifactual error. Physiologic explanations include resorption of a gestational sac and formation of a blighted ovum or a fetus papyraceous. Resorption and formation of blighted ova appear to be quite common, but the formation of a fetus papyraceous is a very rare event (one in 12,000 live births and one in 184 twin births).⁵

Table II. Vanishing twins—group with suspected diagnoses

Patient	Indication for scan	Gestational age at first scan (wk)	Findings	Gestational age at next scan (wk)	Findings	Pregnancy information	Outcome
8	GD	6½	1 sac, + FHM 1 sac, - FHM	9	1 sac, + FHM Echospars area adjacent	Bleeding at 8-9 weeks	Term singleton
9	GD	6	? 2 sacs, - FHM	8½	1 sac, + FHM	Artificial ovulation	Term singleton
10	Hypertension	6½	1 sac, + FHM 1 sac, - FHM	10	1 sac, + FHM	—	Term singleton
11	GD, R/O twins	7	1 sac, ? + FHM 1 empty sac	10½	1 sac, + FHM	Artificial ovulation	Term singleton
12	GD	8½	1 sac, + FHM 1 sac, - FHM	9½	1 sac, + FHM 1 empty sac	Bromocryptine	Term singleton
13	GD	8½	1 sac, + FHM ? 1 empty sac	19	1 viable fetus	—	Death at 37 weeks
14	GD, Infertility	8	1 sac, + FHM ? 1 empty sac	—	—	Artificial ovulation	Term singleton
15	GD	8	1 sac, + FHM 1 small sac	12	1 sac, + FHM 1 small sac	—	Term singleton
16	GD	10	1 sac, + FHM ? 1 empty sac	23	1 viable fetus	—	Term singleton
17	R/O twins	14	1 sac, + FHM 1 empty sac	—	—	—	Term singleton
18	GD	7½	4 sacs, + FHM 1 sac, ? + FHM	8½	4 sacs, + FHM 1 empty sac	Artificial ovulation	Preterm quadruplets
19	GD	7½	2 sacs, + FHM 1 fluid-filled sac	22	2 viable fetuses	Artificial ovulation	Term twins
20	GD	8½	2 sacs, + FHM 1 empty sac	12½	2 sacs, + FHM	—	Term twins
21	GD, bleeding, myomas	8	1 sac, + FHM 1 sac, - FHM	12	1 sac, - FHM	Spotting at 6-13 weeks	Death at 13 weeks
22	R/O ectopic	8½	2 sacs, - FHM	9½	1 sac, + FHM 1 sac, - FHM	—	Death at 11½ weeks
23	R/O ectopic	7	1 sac, + FHM ? 1 empty sac	—	—	—	Elective abortion
24	R/O twins	6	1 sac, + FHM 1 sac, - FHM	—	—	—	Elective abortion

See Table I for key to abbreviations.

False interpretation of either physiologic conditions associated with pregnancy or technical ultrasound artifacts can incorrectly imply the presence of an additional gestational sac on an early sonogram. The normal and changing physiologic cavities that are present early in pregnancy need to be considered, including the amniotic cavity, chorionic sac, extraembryonic coelom, and yolk sac.^{1,6,7} Other physiologic conditions with the potential for overdiagnosing a multiple gestation include an intrauterine collection of blood at the site of the trophoblast in a singleton pregnancy⁸ or in an abnormally progressing pregnancy,³ hydropic change in chorionic villi,³ or a decidual reaction in the second horn of a bicornuate uterus.¹

Artifactual errors are known to distort ultrasonographic images and need to be recognized early to minimize false interpretations.¹ A recent report by Buttery and Davison⁹ describes the ghost artifact, which results in image duplication or triplication. This can be seen especially when the echogenic source is small, such as an early gestational sac, and the distance between

the echogenic source and the transducer is large.⁹

In this study, patients were divided into three groups based on the likelihood that the ultrasonic findings represented a disappearing fetus. It appears likely that a true representation of a vanishing fetus can be seen in the patients within the group with documented diagnoses, in which two viable fetuses were seen on an early sonogram, but subsequent scan could confirm only one viable gestation. Although observer error and ghost artifact cannot be excluded, this group of patients most clearly demonstrates evidence of the vanishing twin. The patients in the group with suspected diagnoses possibly represent cases of disappearing fetuses. Given that an additional empty or abnormal sac was seen in each pregnancy, these findings may represent sonographic evidence of the vanishing process after it has already begun. Considering those cases in the group with doubtful diagnoses, in which a viable gestation was seen within a bilobed sac, explanations for the abnormal shape of the sac, other than possibly representing a twin pregnancy, may include the following: distortion

Table III. Vanishing twins—group with doubtful diagnoses

<i>Patient</i>	<i>Indication for scan</i>	<i>Gestational age at first scan (wk)</i>	<i>Findings</i>	<i>Gestational age at next scan (wk)</i>	<i>Findings</i>	<i>Pregnancy information</i>	<i>Outcome</i>
25	GD, myomas	7	1 bilobed sac, + FHM	16½	1 viable fetus	Artificial ovulation	Term singleton
26	GD, bleeding	7½	1 bilobed sac, + FHM	10	1 sac, + FHM	Spotting at 7 weeks	Term singleton
27	GD	7	1 bilobed sac, + FHM	18	1 viable fetus	—	Term singleton
28	GD	8½	1 bilobed sac, + FHM	10	1 sac, + FHM 1 empty sac	Artificial ovulation; spotting at 7 weeks	Term singleton

See Table I for key to abbreviations.

resulting from excessive pressure applied to the transducer on the patient's abdomen, extrinsic compression from a full bladder, myoma, transient uterine contraction, or an hourglass shape that may represent a normal variant.

The disappearance rate obtained from the present study (21.2%) is based on data obtained only from those patients forming the group with documented diagnoses. This disappearance rate is comparable to reported spontaneous fetal loss rates for singleton pregnancies. Although the true loss rate is unknown, the background rate of spontaneous abortion in the first trimester of pregnancy has been reported as being approximately 10% to 15%.¹⁰⁻¹² A recent study reported a fetal loss rate of 19% in 42 women scheduled to undergo diagnostic chorionic villus biopsies.¹³

As has been found in previous reports and confirmed by the present study, the only apparent complication associated with the disappearance of a fetus is vaginal spotting or bleeding.^{1, 5, 8-10} Indeed, 25% of patients with the possibility of a vanished fetus experienced first-trimester bleeding. The incidence of first-trimester bleeding of the 54 patients in this study was 18.5%. In contrast, of the remaining 946 patients, first-trimester bleeding was reported in only 7.2%.

If a pregnancy is found to be associated with a disappearing fetus, the prognosis for continuing the pregnancy to a point where fetal viability is assured appears to be good.¹ There were only four cases of further fetal death in the group of 28 patients suspected of having a vanishing fetus (14.3%), and two of these four pregnancies had first-trimester bleeding. In all, of the seven patients who had a vanishing fetus associated with first-trimester bleeding, two patients had spontaneous first-trimester losses (28.6%). These data confirm standard obstetric teaching that a pregnancy complicated by first-trimester bleeding is associated with an increased risk of spontaneous loss or other complications.¹⁴

The incidence of multiple gestation ranges from 3.29% to 5.39%, depending on inclusion of some or all of the patients felt to have a vanishing fetus. These

rates are considerably higher than previously quoted, even if one considers the most conservative estimate that represents only those patients in the group with documented diagnoses. According to the literature, the frequency of monozygous twinning is fairly constant at approximately four per 1000 births (0.4%), and the frequency of dizygous twinning ranges from three per 1000 births in women <20 years of age to 14 per 1000 births at ages 35 to 40,¹⁵ increases with the mother's parity, and varies according to race. Our data indicate the incidence of multiple gestation is significantly greater than has been suggested. This may reflect the large number of patients with infertility and ovulation induction at our institution.

The use of artificial ovulants is known to increase the incidence of multiple gestation. The incidence of multiple conception ranges from 6.8% to 17% after treatment with clomiphene citrate and from 18% to 53.5% after treatment with gonadotropins.¹⁶ Use of prolactin-inhibiting agents is not associated with an increased incidence of multiple pregnancies.¹⁵ A recent report by Schneider et al.⁴ reviewed 78 twin pregnancies diagnosed in the first trimester by ultrasound and concluded that early ovular resorption occurred in 63% of spontaneous and 64% of clomiphene-induced gestations.⁴ Our data indicate that a vanished fetus was associated with ovulation induction in nine of 28 patients (32.1%) but only in two pregnancies in the group with documented diagnoses (28.5%). This suggests a disappearance rate that is much lower than that suggested by Schneider et al.⁴

If a patient were to be subjected to frequent scanning evaluations, the disappearance of a fetus could theoretically be followed. Sonographically a second viable fetus could progress to a sac containing a fetal pole, then to a distorted sac with internal echoes, and next to a separate fluid-filled collection adjacent to the normally progressing pregnancy. Further observation would probably offer no sonographic evidence of the previous gestation as the body's mechanism of resorption continues over time. Similarly it is not unlikely to

find a paucity of confirmatory pathologic evidence in aborted specimens or in placentas at delivery.¹

An interesting point from the present study is that a given ultrasound finding early in gestation may have varying outcomes in different pregnancies. Visualizing two gestational sacs can result in twins, a singleton, or a fetal death. The ultrasonologist is therefore urged to present with caution the ultrasound findings and all their implications.

An intriguing idea relates the phenomenon of the vanishing twin and the development of Rh sensitization. It has been surmised that a disappearing Rh-positive fetus in an unsensitized Rh-negative woman may well account for some cases of Rh sensitization that develop during pregnancy. The mechanism might be spontaneous fetal-maternal bleeding.

In conclusion, the present study confirms data obtained from previous reviews regarding the early diagnosis of multiple gestation. It is apparent that the incidence of multiple gestation in humans is higher than has been previously reported. Our study has obtained a rate of 3.29% to 5.39%, which is considerably higher than earlier reported data. The disappearance of a fetus in a multiple gestation occurs in a significant number of pregnancies, and our data show an incidence of at least 21.2%. This result is not dissimilar to the spontaneous fetal loss rate for singletons reported in the literature. The only apparent complication associated with a disappearing fetus is first-trimester bleeding, which occurred in 25% of pregnancies. This appears to be relatively innocuous for the remaining fetus(es). The data are not significantly different for pregnancies conceived with fertility promoting agents. The potential for varying pregnancy outcomes needs to be considered before making a solid diagnosis of a multiple gestation or a vanishing twin.

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Reduction of urethral pressure in response to stress: Relationship to urethral mobility

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It has been postulated that one factor involved in the pathophysiology of stress urinary incontinence is the sustained decrease in urethral closure pressure in response to a sudden increase in intra-abdominal pressure. Twenty-one patients with stress-related pressure decrease (group 1) and seven patients with stable pressures (group 2) were studied. Although closure pressure in group 1 decreased approximately 27% in response to a single cough in a static setting, when studied dynamically with pressure profiles before and after coughing, there was no pressure decrease seen. The evidence presented suggests that the phenomenon of pressure decrease is in actuality an artifact of measurement and not a true physiologic event. The degree of urethral mobility is related to the perceived pressure decrease. (AM J OBSTET GYNÉCOL 1986;155:20-5.)

Key words: Urethral pressure, stress incontinence, decreased urethral pressure in response to stress

An adequate urethral pressure is necessary for the maintenance of urinary continence. This pressure is equally dependent on striated muscle, smooth muscle-elastic tissue, and a vascular component.¹ The maximum urethral closure pressure has been demonstrated to be decreased in women with stress urinary incontinence and related to the severity of incontinence.^{2,3} Other variables potentially influencing the impairment of the urethral closure mechanism are intrinsic urethral pressure variability and the pressure transmission ratio.⁴⁻⁶

Recently, it has been postulated that another variable is actively involved in the lowering of urethral pressure in women with stress urinary incontinence. Any acute stress will produce a sustained decrease in urethral closure pressure (Fig. 1).^{7,8} The loss of pressure and duration of recovery time are dependent on the magnitude and number of stresses applied. Possible explanations for this phenomenon include expulsion of blood from the suburethral vascular plexus, striated muscle relaxation, smooth muscle relaxation, or a combination of all three factors.

In order to further understand and clarify this phenomenon of urethral pressure relaxation after stress, the following investigation was undertaken.

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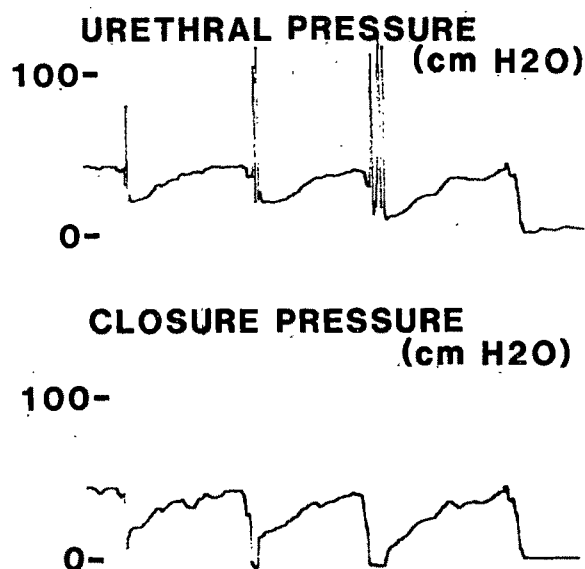


Fig. 1. Urethral pressure decrease in response to one, two, and three coughs, respectively, is demonstrated. The degree of pressure loss and the time necessary for full pressure recovery is related to the magnitude and number of stresses applied.

Material and methods

Twenty-eight consecutive patients undergoing complete urodynamic evaluation for a variety of clinical problems were studied. Detailed history, physical examination, negative urine culture, cystometry, cystourethroscopy, uroflowmetry, and urethral pressure profiles were obtained in all cases. A stress test was performed in the dorsal lithotomy and standing position. The patients were asked to cough, laugh, and occa-

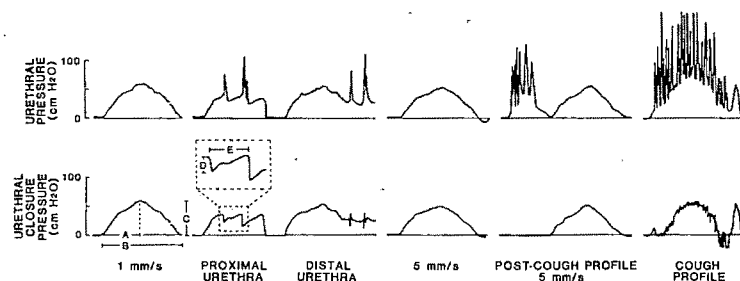


Fig. 2. Urethral pressure profiles were taken at 1 mm/sec, at 5 mm/sec, and after the patient had coughed repeatedly. Distance to maximum closure pressure (A), urethral functional length (B), and maximum closure pressure (C) were recorded from each profile type. The magnitude of pressure decrease (D) and the time necessary for pressure recovery (E) were recorded in the proximal urethra in response to a single cough. In the distal urethra the direction of change was noted (increase, decrease, or no change). A postcough pressure profile was then obtained.

sionally exercise with a full bladder to see if urinary leakage could be documented.

A Q-tip test was performed to measure the degree of urethral mobility.⁹ The patients were placed in the dorsal lithotomy position and a lubricated Q-tip was introduced into the urethra. A resting angle was obtained with the horizontal position used as a reference zero point. The patient was asked to cough and bear down, and the maximum excursion was measured. The angle measurements were obtained with an orthopedic goniometer. Although this technique is in standard use, the precision, accuracy, and normative values have not been well defined.

Urethral, intravesical, and abdominal (vaginal) pressures were obtained simultaneously with microtip pressure transducers,* by methods similar to those previously described.¹⁰ Electronic subtraction was used to obtain urethral closure pressure (urethral minus intravesical) and detrusor pressure (intravesical minus abdominal). Pressures were plotted on a six-channel recorder.† Evaluations were obtained in the supine position with 150 ml of 0.9% saline solution. Variables measured and procedures performed are summarized in Fig. 2.

Three urethral closure pressure profiles were taken by use of a mechanical puller at a withdrawal rate of 1 mm/sec and then repeated at 5 mm/sec. Measurements were recorded from the third profile and all recordings were taken with the microtransducers in the left lateral position. Recorder speed was set to match profilometer speed in each case. Variables recorded included maximum urethral closure pressure, length to peak pressure, and functional profile lengths.

Urethral closure pressure response to stress was eval-

DETRUSOR PRESSURE

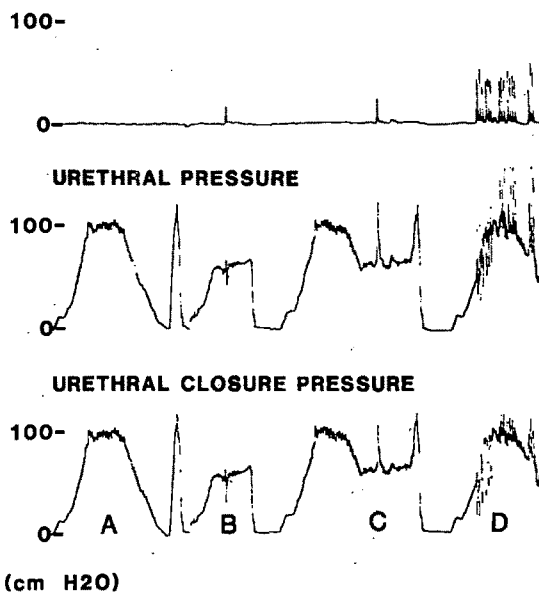


Fig. 3. This tracing of a patient in group 2 demonstrates: A, baseline closure profile; B, no pressure change in the proximal urethra; C, no pressure change in the distal urethra; and D, a negative cough profile.

uated by measuring the magnitude of pressure change and the duration of recovery after a single cough in both proximal and distal urethral segments approximately 0.5 cm from peak closure pressure. A cough urethral pressure profile was then obtained with the patient instructed to cough continuously while the transducer was withdrawn through the urethra.

To further evaluate the extent of the urethral pressure decrease, the patient was asked to cough vigorously 10 times after which a postcough urethral pressure profile at 5 mm/sec was immediately determined. It was previously reported that the pressure decrease appeared to plateau after four to five stresses.^{7,8} In our

*Models PC-630 B and PC-380, Millar Instruments, Houston, Texas.

†Urolab Model 1106, Life Tech, Inc., Houston, Texas.

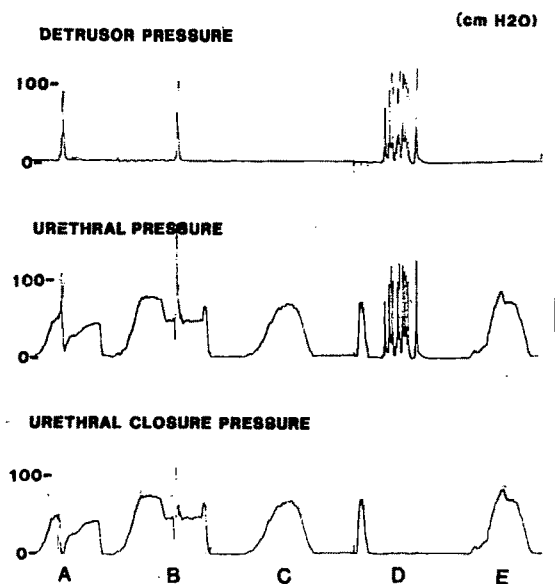


Fig. 4. This tracing of a patient in group 1 demonstrates: A, pressure decrease in proximal urethra in response to cough; B, slight pressure increase in the distal urethra in response to a slightly stronger cough; C, baseline profile at 5 mm/sec; D, repetitive coughing followed by a repeat urethral pressure profile; E, postcough profile demonstrating the decrease in functional length and distance to maximum pressure that was consistently seen in group 1.

own clinical experience, this could vary between two and seven stresses. Ten coughs were selected to ensure that maximum pressure decrease and the corresponding longest possible recovery time had been reached. The objective was to obtain a pressure profile wherein peak pressure was reached in less than 50% of the time necessary for urethral pressure to recover under the previous static measurements of a single cough. Thus, if the urethral relaxation in response to stress was present, maximum closure pressure should be consistently decreased from baseline values.

The condition of stress incontinence was diagnosed on the basis of pressure equalization during a cough profile with simultaneous urinary leakage or a positive stress test in a patient documented to have a stable bladder.

The patients were divided into two groups according to urethral closure pressure response to a single cough in the proximal urethral segment: group 1—pressure decrease in response to stress (Fig. 1); group 2—no change or a pressure increase in response to stress (Fig. 3).

Group 1 consisted of 21 patients. The mean age was 49.4 ± 10.4 years. Mean parity was 2.7 ± 1.5 . Two patients had previously undergone unsuccessful operation for stress urinary incontinence. The primary urodynamic diagnosis included stress incontinence (57%), unstable detrusor (24%), and normal evaluation (19%).

Table I. Urethral closure pressure before and after post cough profiles

Group	Control no. 1 (1 mm/sec)	Control no. 2 (5 mm/sec)	Post cough (5 mm/sec)
1	58 ± 26	58 ± 24	59 ± 37
2	$81 \pm 27^*$	77 ± 31	76 ± 31

*Group 1 (1 mm/sec) with group 2 (1 mm/sec): $p < 0.05$.

The normal examinations were in patients having urodynamic assessments before radical hysterectomy.

Group 2 consisted of seven patients with a mean age of 42.6 ± 15 years. The mean parity was 2.9 ± 2 . In two patients the diagnoses were unstable bladder and sensory urgency, respectively. Three patients had normal examinations before undergoing radical hysterectomy. There were no patients with stress incontinence in this group.

The following statistical analyses were performed: Wilcoxon matched pairs test, a Mann-Whitney U test, and Spearman rank correlation.

Results

In group 1, the average pressure drop in the proximal urethra was 15.3 ± 8.4 cm/H₂O with a mean recovery time of 9.1 ± 4.2 seconds. This pressure drop represents a 27% decrease from baseline maximum closure pressure. Recovery time and pressure drop were not significantly different between patients with stress urinary incontinence and those with other urodynamic diagnoses.

The pressure reduction in group 1 was not consistently seen in the distal urethral segment (Fig. 4). A pressure increase was seen in 52% ($n = 11$), there was no pressure change in 33% ($n = 7$), and there was a pressure decrease in 14% ($n = 3$). The pressure increase was consistently less and the recovery time consistently shorter than the corresponding values in the proximal urethral segment.

The different profilometer speeds used in performing urethral pressure profiles did not alter the maximum closure pressure in either group 1 or group 2 (Table I). The only statistical difference between groups was in the closure pressure measurement taken at 1 mm/sec ($p < 0.05$). Although the urethral pressure was lower in patients with genuine stress urinary incontinence (55 ± 25 cm/H₂O, $n = 12$), this was not significantly different from that in patients with other urodynamic diagnoses (71 ± 28 cm/H₂O, $n = 16$).

After prolonged coughing, there was no change in maximum closure pressure in either group (Table I). Fig. 5 is a typical representation of a patient in group 1 with pressure decrease in the proximal urethral segment without a change in pressure in the postcough

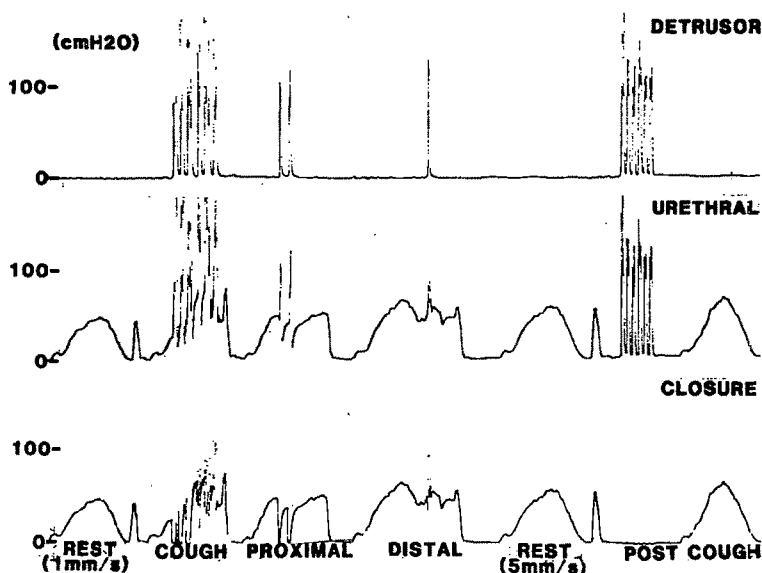


Fig. 5. This patient demonstrates the phenomenon of pressure decrease in the proximal urethra with pressure increase in the distal urethra. Recovery time from a single cough (not shown) was 5.5 seconds. Post-cough profile shows an increase in closure pressure. Elapsed time from last cough to peak closure pressure was 2.8 seconds.

profile. It was felt that the postcough profile was an accurate reflection of events, since the time from the last cough to peak profile pressure averaged 3.5 ± 1 second. (Single cough recovery time was 9.1 ± 4.2 seconds.)

There was no statistical difference in baseline functional length at the various control profilometer speeds (Table II). Although the average baseline functional length in group 2 was increased compared with that in group 1, this was not statistically significant. In the postcough profile, there was a significant decrease in both functional length and distance to peak pressure in group 1. In group 2 there was no significant change in functional length to peak pressure after coughing.

There was a significant difference in the extent of urethral relaxation present between group 1 and group 2. In group 1, the average Q-tip angle change was 39 ± 14 degrees. In group 2 the change was 10 ± 8 degrees ($p < 0.01$). A significant correlation was seen between Q-tip angle change and pressure drop/recovery time in group 1. (Spearman correlation coefficients: pressure drop to Q-tip angle change, $R = 0.48$, $p = 0.013$; recovery time to Q-tip angle change, $R = 0.3838$, $p = 0.41$; pressure drop to recovery time, $R = 0.5034$, $p = 0.01$). The data from which the correlations were drawn are presented in Fig. 6.

Comment

This study demonstrated the following:

1. The urethral pressure decrease in response to stress is present only on static profiles. The pressure

Table II. Functional length and length to peak pressure before and after post cough profiles

Group	Functional length		Length to peak	
	Control (5 mm/sec)	Post cough (5 mm/sec)	Control (5 mm/sec)	Post cough (5 mm/sec)
1	2.6 ± 0.6	$2.3 \pm 0.6^*$	1.5 ± 0.5	$1.2 \pm 0.6^\dagger$
2	2.8 ± 0.5	2.8 ± 0.6	1.3 ± 0.3	1.3 ± 0.3

*Comparison of group 1, control, with group 1, post cough: $p < 0.01$. Comparison of group 1, post cough, with group 2, post cough: $p < 0.001$.

†Comparison of group 1, control, with group 1, post cough: $p < 0.05$.

decrease is not a true physiologic event. Maximum closure pressure in the postcough profile demonstrated no consistent change from that in the resting profiles.

2. The pressure decrease in response to stress appears to be an indicator for patients with stress urinary incontinence. However, 43% of patients presenting with urethral relaxation had other urodynamic diagnoses.

3. In individuals with a pressure decrease in the proximal urethra, there was a pressure increase or no pressure change in 88% of the patients in the distal urethral segment.

4. There was a marked difference in urethral mobility between patients with and those without a pressure decrease. The amount of urethral pressure decrease and the duration of recovery time were significantly correlated with the degree of urethral mobility.

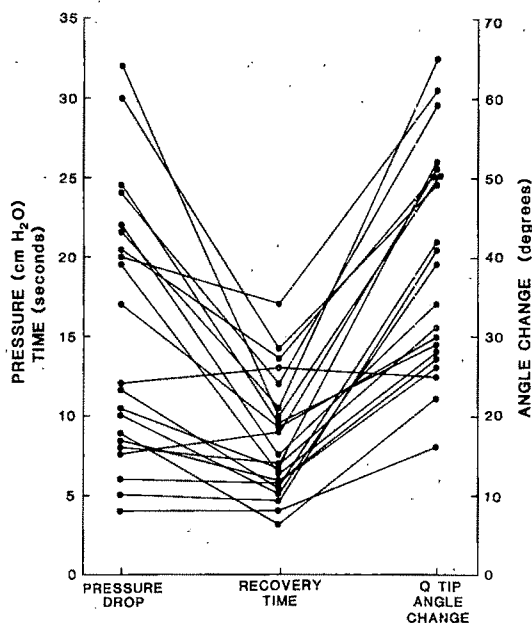


Fig. 6. The relationship in group I between pressure drop, recovery time, and Q-tip angle change is demonstrated. The greater the pressure drop or the recovery time, the greater the angle change observed.

5. In the postcough profile, functional length and length to peak pressure were significantly decreased over control profile measurements.

The observed phenomenon of a urethral pressure decrease in response to stress appears to be an artifact caused by a fixed pressure transducer in the presence of a mobile urethra. The downward and outward rotation of the urethra shifts the stationary microtip sensor toward the urethral-vesical junction. In the proximal urethral segment there is an apparent decrease in urethral pressure as the catheter moves from a high-pressure zone to a low-pressure zone. This same catheter shift in the distal urethra causes movement from a low-pressure zone toward urethral peak pressure. After abdominal stress (coughing) the urethra returns to its original location. In the postcough profile this results in the catheter moving out as the urethra moves in, producing an apparent decrease in functional length and a decrease in the distance to peak pressure.

It has long been recognized that urethral descensus from the symphysis pubis is an important aspect in the pathophysiologic features of stress incontinence and that this mobility results in a decreased abdominal pressure transmission to the proximal urethra. Although not all patients with relaxation have stress urinary incontinence, the majority of patients with genuine stress incontinence have relaxation. Traditionally, this anatomic defect has been measured on either a Q-tip test or a cystogram.^{9,11} The observation that urethral pressure decrease is essentially a function of urethral mo-

bility at times of acute stress opens the possibility of a urodynamic method of quantifying urethral relaxation.

Individuals with a pressure decrease in response to stress in the proximal urethra generally were found to have a pressure increase or no change in pressure in the distal urethral segment. This difference can be explained by the fact that the urethra perforates the urogenital diaphragm approximately 0.5 cm from maximum urethral pressure.¹² The urethra has increased mobility in its proximal segment, but as it enters the urogenital diaphragm it becomes more fixed in its attachments to the symphysis pubis.

Standard urethrocystometry is performed by placing the transducer at peak closure pressure. In the presence of a mobile urethra, we have noticed marked variations in recorded urethral pressure. This is especially apparent in patients in whom the maximum pressure covers a spatial area of limited urethral length. Slight body movement, breathing, or perineal muscle contractions can produce urethral movement that can mimic so-called urethral instability (change in urethral pressure >15 cm H₂O). It is possible that what is being interpreted as urethral instability in some cases is artifactual and related to urethral mobility. Further investigation will be required to clarify this point.

The measurement of the urethral pressure profile continues to be an integral part of the urodynamic evaluation. The measurement of closure pressure identifies patients with inherent sphincter weakness who have a significantly lower cure rate with incontinence operations than patients with normal pressure. The cough profile provides the physician with objective evidence of genuine stress incontinence and enables the physician to determine the actual amount of pressure transmission. Although pressure decrease in response to stress does not appear to be an independent variable in the pathophysiologic features of stress incontinence, it enables the physician to assess the relative degree of urethral mobility.

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Acceptability of chorionic villi sampling for prenatal diagnosis

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The factors that influence women in choosing between first-trimester chorionic villi sampling and second-trimester amniocentesis for prenatal diagnosis were investigated. Five hundred twenty women of advanced maternal age who had previously undergone prenatal diagnosis by amniocentesis and were delivered of a normal infant were requested to complete a questionnaire concerning their attitudes toward amniocentesis and chorionic villi sampling. The majority of respondents indicated that the time at which chorionic villi sampling is performed (76%), the rapid availability of diagnostic results (72%), and the type of abortion procedure available (68%) would make them choose this method. In contrast, the factors that influenced women to choose amniocentesis included the known low risk of spontaneous abortion (76%) and confidence in the skill of the obstetrician who would perform the procedure (56%). When all factors were considered together, 68% of the respondents chose amniocentesis based on the known low risk of spontaneous abortion, whereas for those who chose chorionic villi sampling (32%), the major criterion was the fact that the procedure is performed in the first trimester. However, 87% of women who preferred amniocentesis indicated that if the risk of spontaneous abortion associated with chorionic villi sampling were equal to or less than that of amniocentesis, they would choose chorionic villi sampling. These results indicate that for many women of advanced maternal age, the acceptability and the use of chorionic villi sampling will be dependent on the demonstration that the risk of fetal loss is low, approaching that of amniocentesis. (*AM J OBSTET GYNECOL* 1986;155:25-9.)

Key words: Chorionic villi sampling, amniocentesis, prenatal diagnosis

Recently a new method of obtaining fetal tissue for prenatal diagnosis, called chorionic villi sampling, has been developed.^{1,2} This procedure, which is performed at 8 to 12 weeks of pregnancy, involves the ultrasound-guided transcervical aspiration of a small amount of chorionic villi. This tissue is used directly or can be cultured for diagnostic chromosomal and/or biochemical analyses. Test results are usually available within 2 weeks, thereby providing rapid relief of parental anxiety when the fetus is found normal or, if a fetus with a genetic defect is detected, permitting the parents to choose a safer, easier termination of the pregnancy dur-

ing the first trimester. Preliminary data from 10,000 procedures performed worldwide have revealed that chorionic villi sampling is safe for the mother and that the rate of fetal loss is about 4% when performed by experienced obstetricians. Since most conditions that can be diagnosed by amniocentesis are detectable by chorionic villi sampling, this procedure represents an attractive alternative to amniocentesis, which cannot be safely performed until the second trimester.

Previous studies have documented the safety and accuracy of amniocentesis for prenatal diagnosis.^{3,4} However, experience has identified several disadvantages of the procedure.

Amniocentesis cannot be safely performed until the second trimester of pregnancy, usually during weeks 16 to 18, and the diagnostic results are often unavailable for 2 to 4 weeks after the procedure. Several investigators have reported that the period of waiting for the

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Table I. Sources of stress or anxiety during prenatal diagnosis by amniocentesis

	Response (% of women)					Mean	SD
	1 (none)	2	3 (moderate)	4	5 (extreme)		
Anxiety about a genetic defect being discovered	5	15	61	12	7	2.95	0.80
Anxiety about the procedure itself	14	27	36	11	13	2.94	1.04
Stress during the procedure	36	23	28	4	10	2.33	1.27
Anxiety while waiting for results	6	14	49	15	15	3.12	1.04
Relief after receipt of test results	0	1	13	30	56	4.42	0.76
Overall anxiety during the pregnancy	5	24	38	15	17	2.84	1.20

diagnostic results is the most psychologically stressful aspect of amniocentesis for most women and their partners.⁵⁻⁸ In addition, if the results of the chromosomal and/or biochemical analyses reveal an abnormality and the woman chooses to terminate the pregnancy, a second-trimester abortion must be performed, which carries increased maternal morbidity and mortality, particularly with regard to the psychological trauma.⁹⁻¹¹ Chorionic villi sampling obviates many of the disadvantages associated with amniocentesis, since chorionic villi sampling is performed in the first trimester and the test results are usually available within 1 to 2 weeks.

In this report, the acceptability of chorionic villi sampling versus amniocentesis was investigated by a retrospective study of a population of women who had previously undergone prenatal diagnosis by amniocentesis for the indication of advanced maternal age. Since each of these women had received preamniocentesis genetic counseling and subsequently undergone a successful amniocentesis and were delivered of a normal infant, they were familiar with this method of prenatal diagnosis from which they could compare and subjectively evaluate chorionic villi sampling for fetal diagnoses.

Methods

Patient population. This study included 520 women of advanced maternal age who underwent amniocentesis at the Mount Sinai School of Medicine between April 1, 1983, and March 31, 1984, and who subsequently gave birth to a normal infant. In all of the subjects, advanced maternal age was the only indication for prenatal diagnosis (age-specific risk ranged from 0.4% to 3.2%). The mean age of the women was 36.91 years (range, 34 to 44 years) with a racial distribution of 76%, white; 12%, black; 7%, Hispanic; and 4% Asian. Religious affiliations were 56%, Jewish; 22%, Catholic; 14%, Protestant; and 8%, other or none.

Study design. The name, address, age, marital status,

race, history of previous pregnancies and induced and spontaneous abortions, and religious affiliation were obtained from each patient's record. A questionnaire consisting of 70 open- and closed-ended questions was devised to obtain demographic data and to assess the acceptability of chorionic villi sampling versus amniocentesis for prenatal diagnosis by asking the participants to identify and rank various factors that would influence their choice between chorionic villi sampling and amniocentesis. A questionnaire was mailed to each subject accompanied by a cover letter that requested participation and assured confidentiality and by a description of amniocentesis and chorionic villi sampling. The information provided about each procedure included its possible discomforts, the time at which the procedure is performed, the period of waiting for the diagnostic results, the risk of spontaneous abortion, and the type of abortion that could be performed (if the fetus were affected).

Results

Characteristics of the study population. Of the 520 women who were contacted by mail, 365 completed and returned the questionnaire, resulting in a response rate of 70%. There was no significant difference ($p \geq 0.5$) in the mean age, religious affiliation, or gravidity of the responders and nonresponders to the questionnaire. The mean age of the participants was 37.07 years (± 1 SD = 2.32; range = 34 to 44), 98.3% were married, and 87% had some college or graduate school education. The distribution of religious affiliations were 53%, Jewish; 22%, Catholic; 13%, Protestant; and 12%, other or none. Only 8.6% of the responders had more than one amniocentesis.

Experience with amniocentesis. Each of the respondents was asked about her recent prenatal diagnostic experience with amniocentesis. Eighty-one percent had planned the pregnancy and of these, 50% indicated that the availability of amniocentesis to monitor prenatally

Table II. The influence of various factors on choosing amniocentesis or chorionic villi sampling for prenatal diagnosis

Factor	Response (% of women)		
	"Would make me choose amniocentesis"	"Would make me choose chorionic villi sampling"	"Would make no difference"
Risk of spontaneous abortion	76	8	16
Risk of fetal damage	67	9	24
Confidence in skill of physician	56	3	41
Confidence in test results	39	10	51
Discomfort during procedure	37	8	55
Cost	14	0	86
Time of procedure	9	76	15
Type of abortion available	6	68	26
Period of waiting for test results	5	72	23

the status of the fetus was a factor in their decision to become pregnant. Some respondents first learned about amniocentesis from the media (36%), their obstetricians (36%), or from friends or relatives (14%), whereas others (14%) reported that the availability of amniocentesis was "common knowledge." It was notable that 86% of respondents had sought prenatal care by the eighth week of pregnancy (mean $\pm$ 1 SD = 5.77 $\pm$ 3.13 weeks; range = 1 to 16 weeks), whereas only 37% of women received genetic counseling by the eighth week, with most (63%) seeking prenatal diagnosis during the ninth to seventeenth week of pregnancy (mean $\pm$ 1 SD = 9.48 $\pm$ 3.90 weeks; range = 1 to 18 weeks), presumably reflecting the fact that amniocentesis is usually performed after the sixteenth week of pregnancy.

The major concerns of the respondents before amniocentesis included fear that the test would reveal an abnormality (86%), distress over the possibility that they may have to consider termination of the pregnancy (47%), concern that the fetus would be injured during the procedure (47%), worry that the procedure would cause a spontaneous abortion (37%), and fear of procedure-related discomfort or pain (28%). After amniocentesis the only reported complications included cramps and/or bleeding for several hours (2% of women). The respondents indicated that they received the results of the chromosome studies in 2 to 7 weeks, with an average wait of 3.24 weeks ($\pm$ 1 SD = $\pm$ 1.07 weeks). Seventy-nine percent of respondents stated that they would have terminated the pregnancy if an abnormality had been discovered, whereas 19% were undecided and only 2% would have continued the pregnancy.

The amount of stress and anxiety experienced before, during, and after the amniocentesis and the amount of relief experienced after the diagnostic results were known were assessed. As summarized in Table I, 81% reported that they were only somewhat (that is, moderately or less than moderately) anxious

that their baby might be affected with a genetic disease. Seventy-seven percent of respondents indicated that they were somewhat anxious before amniocentesis, and 86% found the procedure somewhat stressful. In contrast, 83% were moderately or more than moderately anxious during the period while awaiting results, and all but two respondents reported moderate to extreme relief after the diagnostic results were known. Overall, prenatal diagnosis was described as a moderately anxious experience.

Attitudes toward prenatal diagnosis in future pregnancies. Ninety-three percent of respondents indicated that they would definitely seek prenatal diagnosis in a future pregnancy, but the others were undecided. In addition, when asked if they would consider chorionic villi sampling instead of amniocentesis, 45% indicated that they would consider chorionic villi sampling, 20% would not, and 35% were undecided. Respondents were asked to consider separately a series of nine factors and to indicate how each factor would influence their choice between amniocentesis or chorionic villi sampling (Table II). The factors that influenced most respondents to choose amniocentesis included the known low-risk of spontaneous abortion or fetal damage and confidence in the physician's skill in performing the procedure. In contrast, the performance of the procedure during the first trimester of pregnancy, the shorter period of waiting for the diagnostic results, and the type of abortion procedure available in the first trimester were the factors that would influence most of the women to choose chorionic villi sampling. Factors that were not important in choosing between chorionic villi sampling and amniocentesis included the discomfort or pain experienced during the procedure, confidence in the accuracy of the test results, and the cost of the procedure.

When asked to consider all of the factors together and to indicate which procedure they would choose if faced with the decision, 68% of the respondents chose amniocentesis and 32% chose chorionic villi sampling.

Table III. Identification of the critical factors in choosing between amniocentesis or chorionic villi sampling

Factor	Choice indicated by respondent (% of women)	
	Amniocentesis	Chorionic villi sampling
Risk of fetal damage	51	8
Risk of spontaneous abortion	47	4
Confidence in test results	41	4
Confidence in the skill of physician	25	7
Discomfort during procedure	19	0
Type of abortion available	11	0
Time of procedure	9	44
Period of waiting for results	9	69
	7	11

The major factors involved in this decision are summarized in Table III. Respondents who chose amniocentesis indicated their decision was based on the known low risk of damage to the fetus (51%) or risk of spontaneous abortion (47%) and confidence in the test results (41%). The major factors that influenced the respondents to select chorionic villi sampling were the time at which the procedure is done (69%), the type of abortion procedure available (44%), and the shorter waiting period to obtain the results (11%). Of interest was the finding that most respondents who chose amniocentesis indicated that they would consider (47%) or choose (40%) chorionic villi sampling if the risk of spontaneous abortion resulting from this procedure were shown to be no more than that of amniocentesis.

Since the women who participated in this study had previously undergone amniocentesis, it was likely that this experience influenced their attitudes about prenatal diagnosis and the choice between amniocentesis and chorionic villi sampling for a future pregnancy. To determine whether stress and anxiety experienced in the previous prenatal diagnosis significantly affected the choice between the two procedures, a χ^2 analysis of the data was performed. Among respondents who reported that amniocentesis was "extremely stressful" there was a statistically significant increase in selecting chorionic villi sampling over respondents who found amniocentesis to be moderately or not at all stressful ($0.05 > p > 0.02$; $\alpha = 0.05$). In contrast, those respondents who waited a relatively longer time (>3 weeks) to receive diagnostic results did not preferentially choose chorionic villi sampling when compared to those who had waited a shorter period (<3 weeks) for the

test results ($0.50 > p > 0.10$). Similarly, the educational level of the respondents (that is, high school versus college versus graduate school) did not significantly influence their choice ($0.50 > p > 0.10$; $\alpha = 0.05$).

Comment

Health care providers have become increasingly aware of patient consumerism and the impact of patient preferences on the use of diagnostic techniques, participation in screening programs, and compliance with treatment regimens. Thus the comparison of the acceptability of chorionic villi sampling versus amniocentesis to women at an increased risk of having a child with a genetic defect is important for estimating the future use of chorionic villi sampling for prenatal diagnosis. In addition, the identification of the critical factors that influence women choosing between chorionic villi sampling and amniocentesis may provide the basis for the further development and improved delivery of both techniques. Therefore a retrospective study of 365 women of advanced maternal age who had previously undergone amniocentesis was conducted to assess the attitudes of potential users toward chorionic villi sampling and to determine its acceptability compared to that of amniocentesis.

The demographic characteristics of the participants were similar to those reported for patients seeking prenatal diagnosis at other testing centers in New York City and in other areas of the United States.¹²⁻¹⁵ The concerns of the respondents before undergoing amniocentesis in their most recent pregnancy included fear that the fetus would be harmed, that a spontaneous abortion would occur, or that an abnormality might be discovered. These concerns were similar to those reported by Doherty and Rice¹³ for a population who sought prenatal diagnosis in Rochester, New York. In addition, the respondents reported a high level of anxiety during the period of waiting for diagnostic results after amniocentesis (79% reported moderate to extreme anxiety) and a sense of relief after being informed that the chromosomal studies were normal (99% reported moderate to extreme relief). These findings were similar to those observed by other investigators.⁵⁻⁶ The respondents in the present study also had similar concerns and anxieties before, during, and after amniocentesis and a comparable knowledge of the procedure when compared to participants in previous studies of amniocentesis.⁵⁻⁷ Thus it was of interest to determine whether these women would choose amniocentesis or chorionic villi sampling for future prenatal diagnoses and to identify the factors that influenced the women in their choice.

Most respondents indicated that the time at which the procedure is done, the shorter period of waiting for diagnostic results, and the type of abortion available

would make them choose chorionic villi sampling (Table II). In contrast, most respondents indicated that the lower incidence of spontaneous abortion after amniocentesis and their confidence in the obstetrician's skill in performing the procedure would influence them to choose amniocentesis. For 86% of respondents, cost was not an important factor. When all factors were considered together and the women were asked to make a final choice between chorionic villi sampling and amniocentesis, 68% chose amniocentesis. For most respondents this decision was based on the known low risk of spontaneous abortion resulting from amniocentesis and the concern that chorionic villi sampling may in some way harm the fetus (Table III). Of interest was the fact that the majority of respondents whose final choice was amniocentesis indicated that they would consider or choose chorionic villi sampling, provided that the risk of spontaneous abortion resulting from chorionic villi sampling were less or equal to that associated with amniocentesis.

For respondents whose final choice was chorionic villi sampling, the major influencing factors included the time at which the procedure was done and the type of abortion procedure available. Of interest was the finding that only 11% of the women said that the shorter period of waiting for the test results was a factor in their decision to choose chorionic villi sampling. Therefore, despite the fact that numerous studies⁵⁻⁷ have documented the extreme stress that women experience while awaiting for diagnostic results, this did not appear to be a critical factor in selecting chorionic villi sampling rather than amniocentesis.

Thus the respondents who chose amniocentesis were most concerned with the safety of the fetus, whereas women who chose chorionic villi sampling were most concerned with the time at which the procedure was done and the type of abortion procedure available. It was possible that the relative concerns of these two groups of women were determined by their ages, with older women more concerned with safeguarding the continuance of the pregnancy or by their past history of spontaneous abortions. However, the two groups did not differ significantly in either of these parameters (average age = 36.91 versus 36.78 and average number of spontaneous abortions = 0.41 versus 0.38 for those who chose amniocentesis and chorionic villi sampling, respectively). Another possible difference between the two groups, which was not assessed in this study, was the prior history of fertility and ease of conceiving, since women who have experienced fertility problems may be less willing to risk a spontaneous abortion.

Since women of advanced maternal age represent the majority of women seeking prenatal diagnosis, the findings of this study provide insight into the relative ac-

ceptabilities of chorionic villi sampling and amniocentesis. However, women who are at risk for having a child with a genetic defect and are at a higher risk (for example, when both members of a couple are carriers of a recessive disorder) may be more concerned with the timing of the procedure and the type and safety of the abortion procedure available should they elect to terminate an affected pregnancy. Thus it is likely that the acceptability of chorionic villi sampling to such individuals will be greater than for women in whom advanced maternal age is the only indication. In addition, the availability of chorionic villi sampling and the fact that this method permits termination of an affected pregnancy in the first trimester may result in use of this service by women who in the past have not sought prenatal diagnosis by amniocentesis because of religious and moral objections to second-trimester induced abortion.

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Pemphigus in pregnancy: A reevaluation of fetal risk

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Pemphigus vulgaris, a bullous dermatosis, rarely occurs during pregnancy. Previous reviews have suggested that pemphigus has no significant effect on pregnancy. A case report of pemphigus occurring during pregnancy and resulting in an intrauterine death is presented. A reevaluation of the literature indicates that maternal pemphigus is associated with a significant risk of fetal mortality. (AM J OBSTET GYNECOL 1986;155:30-3.)

Key words: Pemphigus, bullous dermatoses

Pregnancy, a period of unique metabolic and endocrine changes, has been associated with both physiologic alterations in the skin as well as a variety of pathologic dermatoses.¹ Pemphigus vulgaris is an uncommon bullous dermatosis occurring most frequently in persons between 40 and 60 years of age.² As such it rarely occurs during pregnancy. Nevertheless, 28 cases of pemphigus during pregnancy have previously been reported in the literature. A recent review of these cases concluded that "pemphigus has no significant effect on pregnancy" and that "neonatal mortality does not appear to be increased."³ We recently managed an additional case of pemphigus in a pregnant woman, which resulted in the birth of a stillborn infant. Prompted by this outcome, we reanalyzed the pertinent literature. Our case report and subsequent discussion of the pathogenesis of maternal and fetal manifestations suggest that pemphigus, similar to certain other bullous dermatoses of pregnancy, may be associated with a significant risk of fetal mortality.

Case report

A 19-year-old primigravid black woman was referred to the obstetric clinic of the Harbor-University of California, Los Angeles Medical Center at 20 weeks' gestation for evaluation of oral erosions and blisters believed to be herpetic in origin. The patient was previously in excellent health, taking no medications with the exception of prenatal iron and vitamins. The patient reported a 6-week history of facial rash, as well as oral blisters and erosions (Fig. 1), which inhibited her ability to eat and drink. Lesions began as clear blisters that subsequently became pustular and ulcerated (Fig.

2). At the time of presentation the patient had noted extension of the lesions to the trunk and abdomen. There was no prior patient or family history of similar dermatoses. The patient was immediately referred to the Division of Dermatology for diagnostic evaluation. Histopathologic examination of representative skin lesions revealed the classic findings of pemphigus vulgaris, including suprabasilar vesicle formation with intraepidermal and intraepidermal acantholytic cells. In addition, both direct and indirect immunofluorescent findings were consistent with the diagnosis of pemphigus (Fig. 3). The obstetric evaluation revealed a fundal height of 20 cm with fetal heart tones. An ultrasound scan revealed fetal measurements consistent with the gestational age and no evidence of anomalies. Because of exacerbation of the patient's skin findings as well as the diagnostic biopsy findings, the patient was admitted to the hospital and therapy initiated with prednisone, 200 mg/day, a high-potency topical steroid, and dicloxacillin, 1 gm/day, to control secondary bacterial infection. Despite initial improvement, the subsequent hospital course was marked by continued development of new blisters and erosions. On hospital day 18 the prednisone dose was increased to 300 mg/day. Shortly thereafter the patient was discharged from the hospital and followed on an outpatient basis. Of note during this period was the fact that the patient had a positive cervical culture for both cytomegalovirus and herpes simplex virus. After discharge from the hospital the patient continued to develop new lesions on an intermittent basis. Because of lack of response to high-dose steroids, azathioprine, 150 mg/day was added to the medication regimen. Prenatal care revealed a progressive size/date discrepancy despite active fetal movement. At 25 weeks' gestation the patient noted a diminution in fetal movement. Ultrasound revealed evidence of an intrauterine fetal death. The patient was hospitalized for induction of labor and delivered a stillborn female infant weighing 780 gm. Autopsy revealed a severely macerated fetus with generalized autolysis. Sections of lung revealed numerous cytomegalovirus inclusions consistent with the gross picture of cytomegalovirus pneumonitis. Other fetal organs and the

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Fig. 1. Whitish eroded tongue plus crusting and erosions on lips.

placenta were unremarkable except for autolysis. Because of severe maceration, no typical skin lesions of pemphigus could be seen on the fetus. However, immunofluorescent studies of fetal skin obtained at delivery revealed intercellular deposition of immunoglobulin G, consistent with pemphigus (Fig. 4).

The patient was discharged on the second postpartum day and was maintained on her previous regimen of prednisone and azathioprine. Over the subsequent year her dosage of prednisone was gradually decreased, punctuated by periodic exacerbations of her skin manifestations. She has now been free of skin lesions for 3 months and her prednisone dosage has been tapered to 5 mg every other day, while the dosage of azathioprine has remained 150 mg/day. However, multiple serious complications of the therapy have occurred, including features of iatrogenic Cushing's syndrome, cataract formation, aseptic necrosis of the right femoral head, and amenorrhea.

Comment

Pemphigus is an intraepidermal bullous disease, which has been classified into four types, of which pemphigus vulgaris is the most severe. In all forms of pemphigus the disruption of cohesive forces between epidermal cells leads eventually to the breaking of intercellular bridges, separation of epidermal cells from each other (acantholysis), and the development of intraepidermal vesicles and bullae. Appearing on previously normal skin or mucous membranes, these lesions have little tendency to heal without treatment. Before use of corticosteroid therapy, mortality from pemphigus was nearly 100%.⁴ Although currently the mortality for pemphigus is approximately 10%,² patients requiring high doses of systemic corticosteroids have a mortality of up to 75%.^{5,6} The cause of death is most often secondary infection of large denuded areas and subsequent bacteremia.⁷

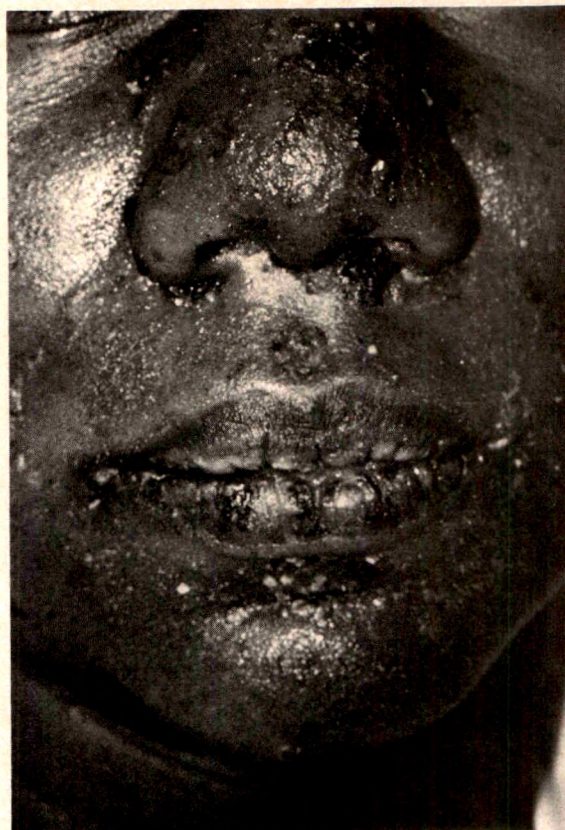


Fig. 2. Multiple crusted erosions on face.

Immunopathologic examination of pemphigus patients has identified serum autoantibodies that react with an intercellular substance of stratified squamous epithelium, demonstrable by indirect immunofluorescent staining.⁸ Direct immunofluorescent staining of perilesional skin almost invariably reveals IgG deposits in the intercellular spaces. IgA, IgM, and complement may or may not be present, and complement is apparently not involved in the pathogenesis of the disease.^{9,10} In the past few years pemphigus has been shown convincingly to be an autoimmune disease.

As with other autoimmune disorders (systemic lupus erythematosus, Graves' disease, immune thrombocytopenic purpura) transplacental transfer of IgG antibodies has been reported in pemphigus. Fetal epidermal lesions consistent with pemphigus, circulating pemphigus antibodies in fetal plasma, and intercellular deposition of IgG in fetal skin have all been reported.¹¹⁻¹⁵ As noted by Kaplan and Callen,³ pemphigus was transferred from affected mother to neonate in 12 of 28 cases, eight infants of which survived. Fortunately, in the surviving infants the bullae and erosions seen at birth are transient, resolving within 1 to 2 weeks.

Our patient represents the twenty-ninth reported case of pemphigus during pregnancy. Of this group, four (14%) have resulted in stillbirths. Wasserstrum and

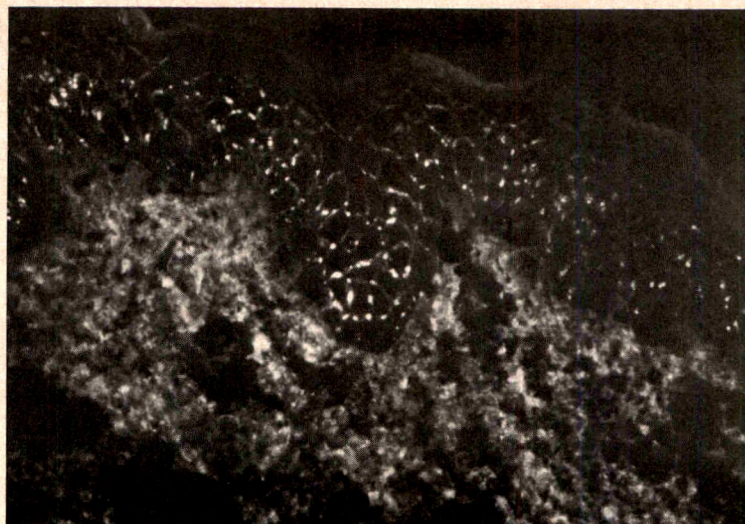


Fig. 3. Direct immunofluorescence of perilesional skin, revealing intercellular staining.

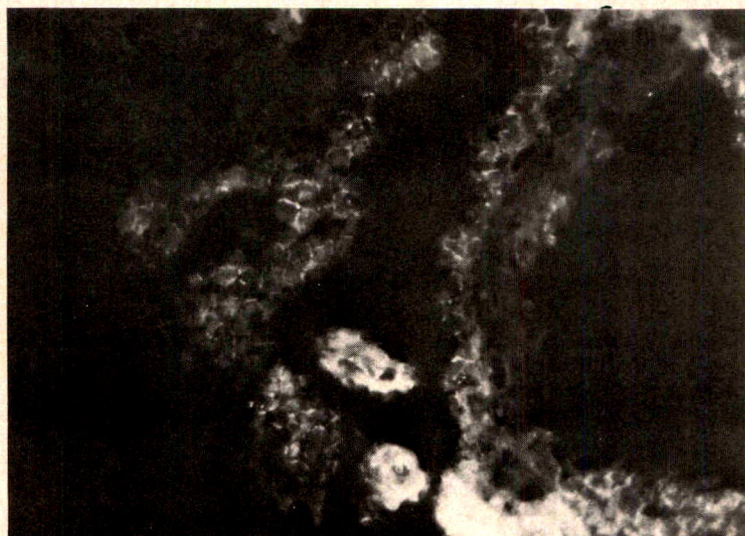


Fig. 4. Immunofluorescent study of fetal skin shows intercellular staining as well as acantholytic cells.

Laros¹⁵ reported the intrauterine death of a 2770 gm male infant despite weekly nonstress tests. Terpstra et al.¹¹ noted the intrauterine death of a severely growth-retarded infant (1116 gm) at 33 weeks' gestation. Green and Maize¹³ reported a spontaneous stillbirth of a 2056 gm female infant. In all of these cases there was evidence of fetal bullous lesions consistent with pemphigus. The fetal death rate of 14% in this group of patients is dramatically elevated above the expected rate of 1%.¹⁶ However, the three reported first-trimester abortions³ among pregnant patients with pemphigus is consistent with the expected incidence. The previous conclusion that the prevalence of fetal and neonatal death is not greater than expected³ appears to be due to use of the incidence of spontaneous

abortions in the normal population as the control rate for fetal death.

In our opinion pemphigus must now be classified together with herpes gestationis and impetigo herpetiformis as dermatoses associated with an increased risk of fetal mortality. The cause of fetal death in the present case appears secondary to maternal and fetal immunodeficiency from high-dose corticosteroids and azathioprine, leading to overwhelming cytomegalovirus pulmonary infection. The cause of fetal death in other cases of bullous dermatosis has not been determined. Mechanisms considered have included placental malfunction,¹¹ maternal steroid administration,^{11, 15} and reaction to autoantibodies.¹³ Death does not appear to be solely a result of fetal bullous disease, since eight

newborn infants with pemphigus lesions have survived and the blisters have resolved.³ Unfortunately, we do not know whether antepartum fetal surveillance can identify those at risk for fetal death. Nevertheless, it is now apparent that pemphigus occurring during pregnancy is associated with a high risk of fetal loss.

We are grateful to Shi-Kaung Peng, M.D., Ph.D., for pathologic examination of skin specimens and immunofluorescence studies.

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A new technique for flexion of the aftercoming head during breech delivery

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A new technique to accomplish flexion of the aftercoming head during breech delivery is described. The technique, used during either abdominal or vaginal delivery, can be performed without an assistant, is useful for large or small infants, and is easily accomplished. (*Am J Obstet Gynecol* 1986;155:33-4.)

Key words: Breech delivery, flexion, aftercoming head

Although recommendations regarding the route of delivery for breech presentation have changed substantially during the past 10 years, techniques necessary to effect a safe, atraumatic delivery are still essential. We often read statements concerning the lack of experience that the house staff has with breech delivery. Yet, to those of us involved with house staff training

or to other experienced practitioners, it is obvious that many of the techniques used in vaginal breech deliveries can also be effectively applied through an uterine incision.

One of the most important principles of breech delivery is the maintenance of flexion of the head during its delivery. Although this can be aided by use of Piper's forceps during vaginal delivery, the Mauriceau-Smellie-Veit maneuver can also be employed, whether delivery is by the vaginal or abdominal route. Described below is a new modification of the Mauriceau-Smellie-Veit maneuver which can be performed without an assistant and which effects easy flexion of the after-coming head. Like the classical maneuver, it can be employed for

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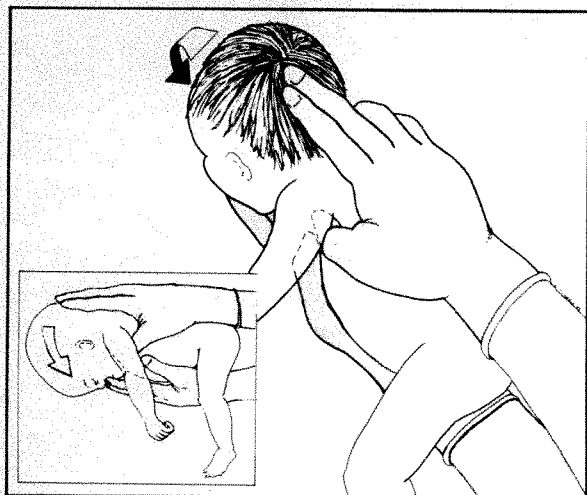


Fig. 1. An oblique view showing the finger placement of the operator's upper hand before flexion. The finger placement of both hands can be seen after flexion has been accomplished (insert).

either vaginal or abdominal delivery and, importantly, is also easily applied to the delivery of infants of very low birth weight.

Technique

The new technique is represented in Fig. 1. After delivery of both arms the infant is placed astride the anterior surface of the lower arm as described with classic technique. The original technique was described as having the forefinger of the lower hand placed in the mouth of the infant, although most authors now agree that placement of the forefinger and middle finger over the upper jaw on either side of the nose is preferable (illustrated in *Williams' Obstetrics*,¹ see insert in Fig. 1). To perform the new technique, placement of the fingers of the upper hand differs from that described by the classic technique. The ring and little finger are placed over one shoulder and the thumb is placed on the other shoulder while the middle and forefingers are slid as far as possible along the back of the neck for term infants or until they reach the occipital protuberance in the small premature infant. Then while simultaneously pushing down with the middle

and forefingers of the upper hand and pushing toward the operator with the same two fingers of the lower hand, adequate flexion of the aftercoming head can be achieved. The technique is identical for either vaginal or abdominal delivery.

Comment

In the latest edition of *Williams' Obstetrics*, the description of the classic Mauriceau-Smellie-Veit maneuver is limited to its application to vaginal delivery only and by its requirement of "moderate suprapubic pressure by an assistant." The finger placement of the operator's upper hand over both shoulders on either side of the neck is described as in the classic application. Flexion is accomplished jointly by use of the lower hand and by the efforts of the assistant.¹ In the 1971 edition of *Munro Kerr's Operative Obstetrics*, the editors have included a brief history of the classic maneuver, from Mauriceau's original description in 1664 to Veit's careful description in 1906. During the description of the classic maneuver in that text, the editors mention a "suggestion" by Wigand that the "middle and fore finger (of the upper hand) *push-up* the occiput" (italics are mine).² No reference was given for this suggestion. Although the finger placement of Wigand's suggested modification is the same as the maneuver described by the current work, the purpose was to "splint" the spinal column in order to avoid excessive traction rather than to accomplish flexion.

Independent of the choice of route of delivery for an infant with a breech presentation, adequate flexion of the aftercoming head must be maintained. The new method described above does not require an assistant, is easily performed on large and small infants, and can be used during vaginal or abdominal deliveries. It is hoped that this useful technique can be added to the current methods and skills of obstetricians.

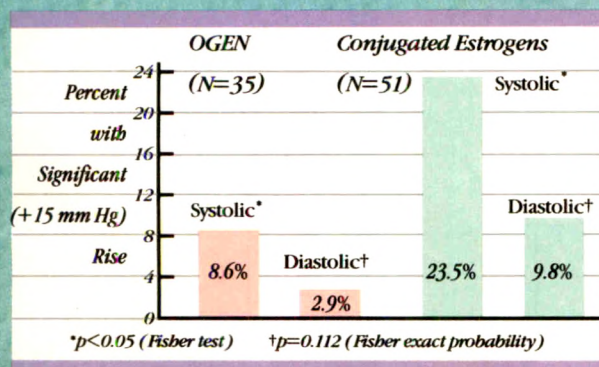
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1. Wren BG, Routledge DA: Blood pressure changes: Oestrogens in climacteric women. *Med J Aust* 2:528-531, 1981.

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WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropirate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropirate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe vasomotor symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIRATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. **Induction of malignant neoplasms.** Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. **Gall bladder disease.** A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. **Effects similar to those caused by estrogen-progestogen oral contraceptives.** There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would

be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. **Thromboembolic disease.** It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. **Hepatic adenoma.** Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. **Elevated blood pressure.** Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. **Glucose tolerance.** A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. **Hypocalcemia.** Administration of estrogens may lead to severe hypocalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. **Fluid retention** — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. **Information for the Patient.** See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. **Drug Interactions.** The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of **broad spectrum antibiotics** which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving **insulin** may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

- a. Increased sulfobromophthalein retention.
- b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability.
- c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T₄ by column, or T₄ by radioimmunoassay. Free T₃ resin uptake is decreased, reflecting the elevated TBG; free T₄ concentration is unaltered.
- d. Abnormal glucose tolerance test results.
- e. Decreased pregnandiol excretion.
- f. Reduced response to metyrapone test.
- g. Reduced serum folate concentration.
- h. Increased serum triglyceride and phospholipid concentration.

D. **Carcinogenesis.** Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. **Pregnancy.** Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. **Nursing Mothers.** Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. **Pediatric Use.** Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. **Genitourinary system.**
 Increase in size of uterine fibromyomata.
 Vaginal candidiasis.
 Cystitis-like syndrome.
 Dysmenorrhea.
 Amenorrhea during and after treatment.
 Change in cervical eversion and in degree of cervical secretion.
 Breakthrough bleeding, spotting, change in menstrual flow.
 Premenstrual-like syndrome.
2. **Breast.**
 Tenderness, enlargement, secretion.
3. **Gastrointestinal.**
 Cholestatic jaundice.
 Vomiting, nausea.
 Abdominal cramps, bloating.
4. **Skin.**
 Hemorrhagic eruption.
 Erythema nodosum.
 Erythema multiforme.
 Hirsutism.
 Chloasma or melasma which may persist when drug is discontinued.
 Loss of scalp hair.
5. **Eyes.**
 Steepening of corneal curvature.
 Intolerance to contact lenses.
6. **CNS.**
 Chorea.
 Mental depression.
 Migraine, dizziness, headache.
7. **Miscellaneous.**
 Aggravation of porphyria.
 Edema.
 Reduced carbohydrate tolerance.
 Increase or decrease in weight.
 Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdose of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. Given cyclically for short-term use:

For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms — One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. Given cyclically:

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropirate tablets, USP) is supplied as OGEN .625 (0.75 mg estropirate), yellow tablets. NDC 0074-3943-04; OGEN 1.25 (1.5 mg estropirate), peach-colored tablets. NDC 0074-3948-04; OGEN 2.5 (3 mg estropirate), blue tablets. NDC 0074-3951-04; and OGEN 5 (6 mg estropirate), light green tablets. NDC 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab[®]) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

5043716/R1

 **Abbott Pharmaceuticals, Inc.**
 North Chicago, Illinois 60064

Outstanding mixed aerobic/anaerobic coverage

Cefizox[®] IM/IV

brand of sterile
ceftizoxime sodium

Injection: 1 and 2 gram vials

- **B.I.D. Dosing[†] for All but Life-Threatening Infections**

- **Clinical Cure Unsurpassed By Clindamycin/Tobramycin[‡]**

A recent, prospective, randomized, comparative trial demonstrated that 'Cefizox' was equal to clindamycin/tobramycin in the treatment of intra-abdominal infections.

- **Better Gram-negative and *Bacteroides* Coverage Than Mefoxin[®] (cefoxitin)[‡]**

Aerobic Organisms ² (number of strains)	CEFIZOX [®] (ceftizoxime) MIC ₉₀	MEFOXIN [®] (cefoxitin) MIC ₉₀
Gram-Negative	mcg./ml.	mcg./ml.
<i>Escherichia coli</i> (40)	0.04	6.3
<i>Klebsiella pneumoniae</i> (32)	0.05	25
<i>Enterobacter aerogenes</i> (16)	0.12	>400
<i>Enterobacter cloacae</i> (16)	0.8	400
<i>Proteus</i> sp. (indole positive) (26)	6.3	12.5
<i>Citrobacter</i> sp. (32)	0.2	400
Gram-Positive	mcg./ml.	mcg./ml.
<i>Staphylococcus aureus</i> (16)	3.1	3.1
<i>Bacteroides</i> species ³ (number of strains)	CEFIZOX [®] (ceftizoxime) MIC ₉₀	MEFOXIN [®] (cefoxitin) MIC ₉₀
<i>B. fragilis</i> (55)	8 mcg./ml.	16 mcg./ml.
<i>B. ovatus</i> (17)	16 mcg./ml.	64 mcg./ml.
<i>B. thetaiotaomicron</i> (13)	64 mcg./ml.	32 mcg./ml.
<i>B. distasonis</i> (12)	8 mcg./ml.	32 mcg./ml.
<i>B. vulgatus</i> (3)	8 mcg./ml.	16 mcg./ml.

**FOR INFECTIONS
BELOW THE DIAPHRAGM
including the septic pelvis***

* Due to susceptible strains of indicated organisms.

† Dosage in renally compromised patients may require adjustment. Please see complete prescribing information.

‡ *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Harding, G.K.M., et al.: Rev. Infect. Dis. 6(Suppl. 1) S283-S292, 1984.

2. Fu, K.P., and Neu, H.C.: Antimicrob. Agents Chemother. 17:583-590, 1980.

3. Aldridge, K.E., et al.: Antimicrob. Agents Chemother. 26:410-413, 1984.

Please consult last page of this advertisement for brief summary of prescribing information.

Cefizox[®]

brand of sterile ceftizoxime sodium and ceftizoxime sodium injection

Before prescribing, see complete prescribing information in SK&F literature or PDR. The following is a brief summary.

INDICATIONS

'Cefizox' is indicated in the treatment of infections due to susceptible strains of the microorganisms listed below:

LOWER RESPIRATORY TRACT INFECTIONS caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Klebsiella* sp.; *Proteus mirabilis*; *Escherichia coli*; *Haemophilus influenzae* including ampicillin-resistant strains; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Serratia* sp.; *Enterobacter* sp.; and *Bacteroides* sp.

URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli*; *Pseudomonas* sp. including *Ps. aeruginosa*; *Proteus mirabilis*; *P. vulgaris*; *Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens*; *Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae*. **INTRA-ABDOMINAL INFECTIONS** caused by *Escherichia coli*; *Staphylococcus epidermidis*; *Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis*; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli*; *Bacteroides* sp. including *B. fragilis*; *Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis*; *Escherichia coli*; *Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis*; *Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis*; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis*; *Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae*. 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae*.

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use. Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox', nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatinine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the most frequent adverse reactions (greater than 1%, but less than 5%) are: **Hypersensitivity**—Rash, pruritus, fever. **Hepatic**—Transient elevation in SGOT, SGPT and alkaline phosphatase. **Hematologic**—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. **Local**—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (less than 1%) are: **Hypersensitivity**—Numbness has been reported rarely. **Hepatic**—Elevation of bilirubin has been reported rarely. **Renal**—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. **Hematologic**—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. **Genitourinary**—Vaginitis has rarely occurred. **Gastrointestinal**—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

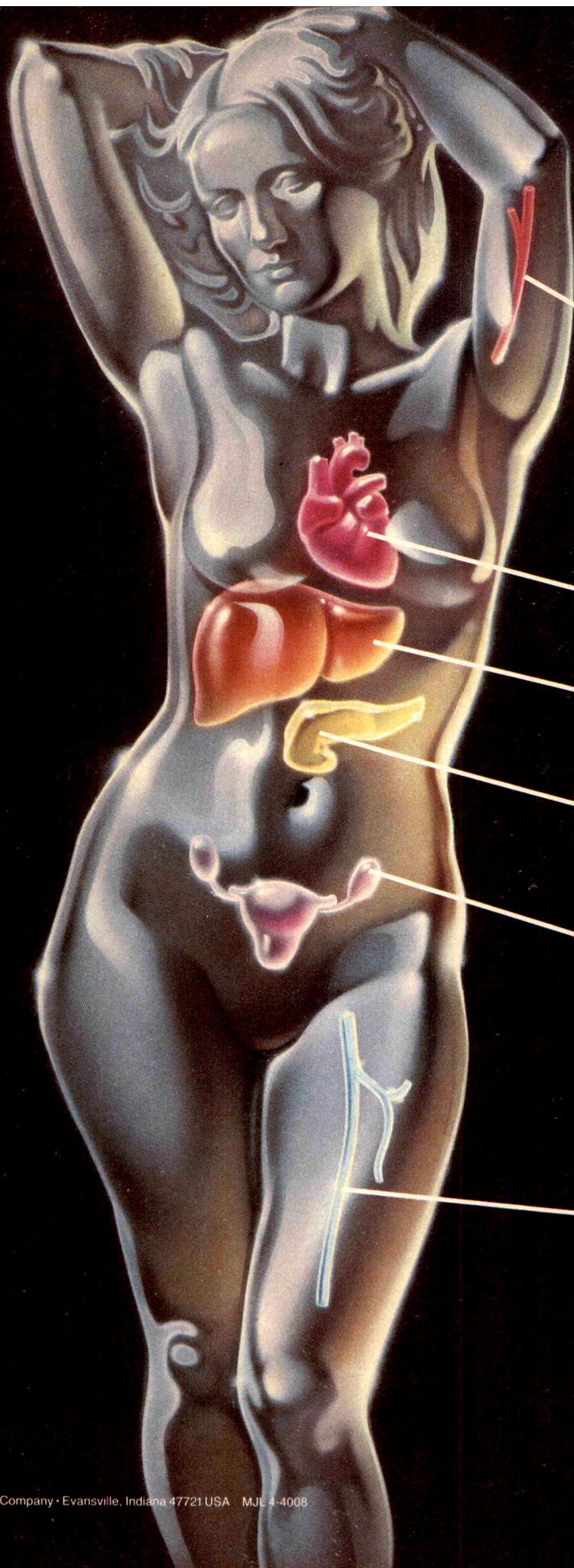
'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

BRS — CF:L14

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Division of SmithKline Beckman Corporation



Manufactured for
Fujisawa SmithKline Corporation
Distributed by
SK&F Laboratories
Division of SmithKline Beckman Corp.



OVCON[®]-35

(norethindrone [0.4 mg] and
ethinyl estradiol [0.035 mg]) tablets

To minimize unwanted oral contraceptive effects...

on lipid metabolism

Serum cholesterol, HDL-C, LDL-C, and
triglycerides remained within normal range^{1-4*}

on blood pressure

Virtually all women studied remained free
of hypertension^{5†}

on liver function

Nearly all liver function tests remained
normal^{6‡}

on carbohydrate metabolism

No significant changes in carbohydrate
metabolism^{7§}

on pituitary and ovarian function

No markedly suppressed pituitary or
ovarian function^{8†}

on coagulation

Coagulation mechanism unaltered^{9*}

*As noted in the labeling required of all oral contraceptives, an increase in triglycerides and total phospholipids has been observed in patients on oral contraceptives. The findings in these studies represent preliminary information and are yet to be confirmed. Prolonged follow-up will be necessary to see if clinical differences in processes such as atherogenesis can be observed in patients receiving OVCON-35.

†As noted in the labeling required of all oral contraceptives, an increase in blood pressure has been reported in patients receiving oral contraceptives. Increased blood pressure may lead to hypertension, defined as sustained pressure greater than 140/90 mm Hg. These data are from the case reports of 13 investigators and were analyzed by Mead Johnson personnel.

‡As noted in the labeling required of all oral contraceptives, certain liver function tests may be affected by estrogen-containing oral contraceptives, including increased sulfobromophthalein retention and increased prothrombin. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives; a few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

§As noted in the labeling required of all contraceptives, a decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The findings in these studies represent preliminary information and are yet to be confirmed. Prolonged follow-up will be necessary to see if clinical differences in processes such as atherogenesis can be observed in patients receiving OVCON-35.

¶It is generally accepted that all combination oral contraceptives act primarily by suppressing the pituitary-ovarian axis. The significance of different degrees of such suppression for the overall safety of any oral contraceptive steroid is not known. The oral contraceptive product prescribed for any patient should be one with the lowest estrogen content compatible with an acceptable pregnancy rate and patient acceptance.

#As noted in the OVCON labeling, a tendency toward increased blood coagulability has been observed in a significant percentage of patients on oral contraceptives. The findings in this present study represent preliminary information and are yet to be confirmed. Prolonged follow-up will be necessary to see if clinical differences in coagulation states can be observed in patients receiving OVCON-35.

References

1. Roy S, Krauss RM, Mishell DR Jr, et al: The metabolic effects of a low-estrogen/low-progestogen oral contraceptive, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive*. New York, Biomedical Information Corp, 1981, pp 24-31.
2. Krauss RM, Roy S, Mishell DR Jr, et al: Effects of two low-dose oral contraceptives on serum lipids and lipoproteins: Differential changes in high-density lipoprotein subclasses. *Am J Obstet Gynecol* 1983;145:446-452.
3. Nolelovitz M, Gudat J, McKenzie LC: Contraceptives, lipids, and lipoproteins: A preliminary report, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive*. New York, Biomedical Information Corp, 1981, pp 20-22.
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7. The effects of a low-estrogen oral contraceptive on carbohydrate metabolism: A review of five studies, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive*. New York, Biomedical Information Corp, 1981, pp 52-61.
8. The effects of a low-estrogen oral contraceptive on pituitary and ovarian secretions: A review of three studies with a graded GnRH stimulation test, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive*. New York, Biomedical Information Corp, 1981, pp 32-38.
9. Nolelovitz M, Kitchens CS, Coone L, et al: Low-dose oral contraceptives: Usage and coagulation, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive*. New York, Biomedical Information Corp, 1981, pp 46-50.

An effective balance of the most trusted estrogen and the best tested progestogen

For Brief Summary, please see last page of this advertisement.

MeadJohnson LABORATORIES

OVCON®-50

(norethindrone [1 mg] and
ethinyl estradiol [0.05 mg]) tablets

OVCON®-35

(norethindrone [0.4 mg] and
ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg, or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg, or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/tokemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg, or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/tokemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and

there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gallbladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) **Ectopic Pregnancy.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breast Feeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin. c. Increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T₄ by column, or T₄ by radioimmunoassay. Free T₃ resin uptake is decreased, reflecting the elevated TBG. Free T₄ concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis, benign hepatomas, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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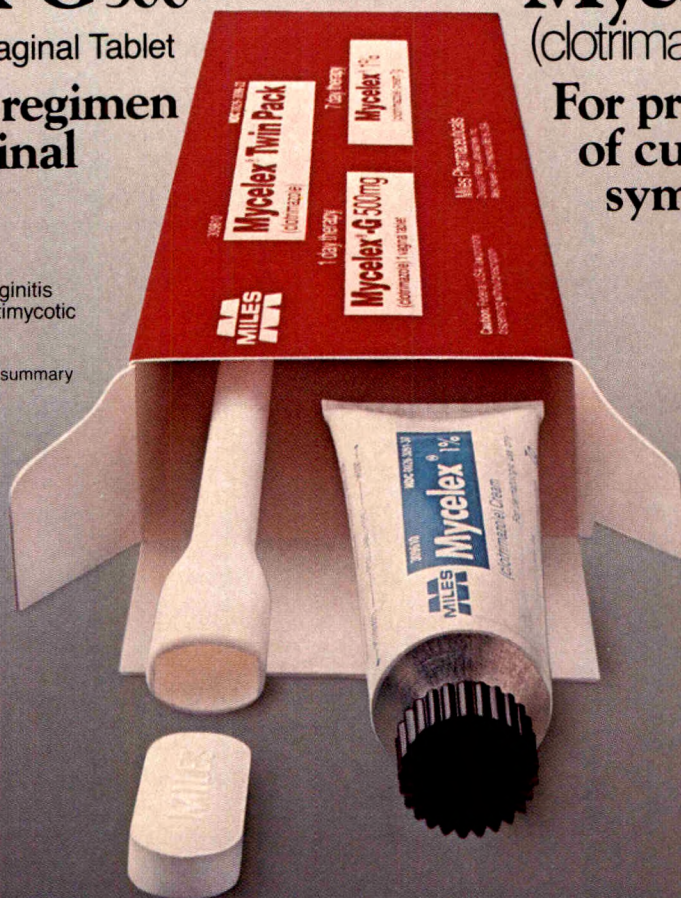
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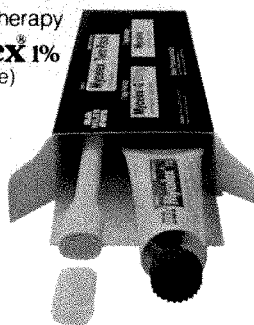
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Precautions

MYCELEX-G 500 mg Vaginal Tablets: If there is a lack of response to the Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

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Carcinogenesis

MYCELEX-G 500 mg Vaginal Tablets: No long-term studies in animals have been performed to evaluate the carcinogenic potential of 500 mg Vaginal Tablets intravaginally. A long-term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

Usage in Pregnancy: Pregnancy Category B:

MYCELEX-G 500 mg Vaginal Tablets: The disposition of ¹⁴C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

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Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy. MYCELEX CREAM: In the first trimester of pregnancy, Mycelex Cream should be used only when considered essential to the welfare of the patient.

Adverse Reactions

MYCELEX-G 500 mg Vaginal Tablets: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

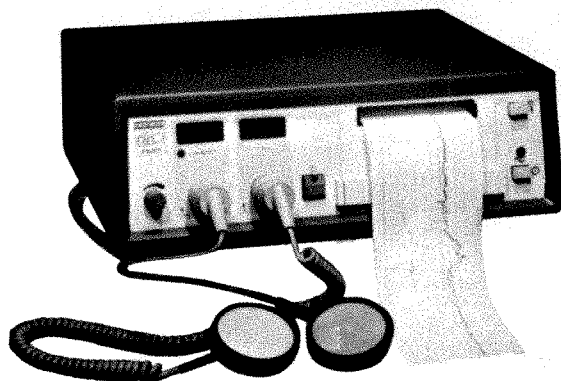
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Recovery of *Chlamydia trachomatis* from the endometrium of women at risk for chlamydial infection

Robert B. Jones, M.D., Ph.D., James B. Mammel, M.D., Marguerite K. Shepard, M.D., and Roberta R. Fisher, M.S.N.

Indianapolis, Indiana

Chlamydia trachomatis is the most common sexually transmitted disease in Western Society today and is a major cause of salpingitis and tubal infertility. However, the frequency with which it produces upper genital tract infection in asymptomatic women has not been determined. Endometrial, endocervical, and urethral cultures for *C. trachomatis* were obtained from 60 women who were at risk for chlamydial infection but who did not have evidence of endometritis or salpingitis on physical examination. Chlamydia was isolated from the lower genitourinary tract in 26 (43%) and from the endometrium in 12 (20%). Thus 12 of 29 (41%) women infected with *C. trachomatis* had endometrial infections. Upper genital infections appear to be common in women at risk for chlamydial infection, and spread to the upper tract may occur shortly after the infection is acquired. (AM J OBSTET GYNECOL 1986;155:35-9.)

Key words: *Chlamydia trachomatis*, chlamydial infections, endometritis

Chlamydia trachomatis is the most prevalent sexually transmissible agent in developed countries today.¹ Its role in nongonococcal urethritis in men and in cervicitis and salpingitis in women seems to be well established.^{1,2} Major and apparently frequent consequences of such infection in women include infertility and ectopic pregnancies.^{1,3,4} The primary site of infection is probably most often the endocervix followed by canalicular spread to the endometrium and fallopian tubes. *C. trachomatis* has been recovered from the endometrium of as many as 44%⁵ and the fallopian tubes of as many as 30%⁶ of women with acute salpingitis.

However, the majority of cases of tubal disease associated with chlamydial salpingitis appear to result from chronic, subclinical infections rather than acute disease,^{3,4} and chlamydial salpingitis tends to be a more subacute process than salpingitis associated with other organisms.⁷

Spread from the endocervix to the endometrium presumably precedes, or occurs simultaneously with, spread to the fallopian tubes. Endometritis due to *C. trachomatis* in association with salpingitis is well documented.^{2,5,8,9} In addition it has recently been shown that women with mucopurulent cervicitis have a high incidence of endocervical infection with *C. trachomatis*,

in association with symptoms, physical findings, and histopathologic features consistent with endometritis.¹⁰

However, the frequency with which chlamydial infections spread from the lower to the upper genital tract is not known, nor is the frequency with which such spread produces symptoms. In order to address these questions, we performed endometrial cultures in a group of women who were at risk for chlamydial infection but who did not have clinical endometritis or salpingitis.

Material and methods

The study population consisted of 60 women who were attending a sexually transmitted disease clinic for evaluation and treatment and who were considered at risk for genital tract infection with *C. trachomatis* because of a recent positive culture for *Neisseria gonorrhoeae* or *C. trachomatis* or because of known sexual contact with a man with gonorrhea, nongonococcal urethritis, or a culture-proved chlamydial infection. Patients were excluded from participation if they were found on examination to have pelvic masses or adnexal, uterine, or cervical motion tenderness. They were also excluded if they were pregnant, had an intrauterine contraceptive device in place, or had received antibiotics in the preceding 14 days. The patients studied were between the ages of 16 and 32. Forty-two of the women were black and 18 white.

After informed consent had been obtained, a bimanual pelvic examination was performed with water used as a lubricant in order to document the absence or presence of tenderness or masses and to ascertain uterine size and position. A speculum examination was then performed. The ectocervix was wiped clear of

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Table I. Presenting diagnosis and culture results in women undergoing endometrial and lower genital tract cultures for *C. trachomatis*

Presenting diagnosis	Positive cultures							
	Lower tract		Endometrium		Both sites		Either site	
	n	%	n	%	n	%	n	%
Gonorrhea	4	36	2	18	1	9.0	5	45
Gonorrhea contact	11	42	5	19	4	15	12	46
Nongonococcal urethritis contact	8	44	4	22	3	17	9	50
Chlamydia contact	2	100	0		0		2	100
Chlamydia infection	2	100	0		0		2	100
Total	26	43	12	20	9	15	29	48

Results given as number with indicated culture result and percent of patients with indicated presenting diagnosis.

Table II. Factors associated with genital and endometrial *C. trachomatis* infections

	Lower genital tract				Endometrium			
	Positive (n = 26)		Negative (n = 34)		Positive (n = 12)		Negative (n = 48)	
	n	%	n	%	n	%	n	%
Past history of STD								
None	19	69	12	30	7	58	24	50
Salpingitis	0		1	2.9	0		1	2.1
Gonorrhea	7	27	19	56	5	42	21	44
Other	0		7	21	0		7	15
Symptoms								
None	15	58	17	50	4	33	28	58
Dysuria	2	7.7	2	5.9	2	17	2	4.2
Pain	2	2.7	1	2.9	2	17	1	2.1
Discharge	9	35	12	35	6	50	15	31
Other	3	7.7	5	8.8	2	17	5	10
Gonorrhea	15	58	18	53	7	58	26	54
Cervicitis								
Absent	14	54	29	85	6	50	37	77
Present	12	22	5	15	6	50	11	23
Mucopurulent	10	38	5	15	5	42	10	21
Gonorrhea	5	50*	2	40*	4	80*	3	30*
No gonorrhea	5	50	3	60	1	20	7	70

STD = Sexually transmitted diseases.

*Percent of women with mucopurulent cervicitis.

mucus with a gauze-covered sponge, and endocervical cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were obtained with the use of rayon-tipped plastic swabs. Cervicitis was diagnosed if severe hypertrophy was present and a diagnosis of mucopurulent cervicitis was made if the endocervical contents were yellow or yellow-green.¹¹ Urethral cultures for *C. trachomatis* were also obtained as previously described.¹² The ectocervix was then washed with Betadine-soaked cotton proctologic swabs and a tenaculum or ringed forceps was used to grasp the anterior cervical lip. An endometrial brush biopsy specimen was then obtained for culture with the uterine sampling device, a double-sheathed wire biopsy culture brush (Medi-Tech, ISO Collidge Ave., Watertown, MA 02172). After the ectocervix had been cleansed, the brush was placed in the endocervical canal

and the inner catheter was advanced through the endocervix and internal os. A protective wax plug was then dislodged and a wire biopsy culture brush was advanced into the endometrial cavity until resistance was met. The brush was rotated to obtain the specimen, then withdrawn to well within the sheath, and the sheath was removed from the uterus. The end of the sheath was cut off and discarded, and the brush was then extruded and placed in transport media for chlamydial culture.

All specimens for chlamydial culture were stored at 4° C for no more than 24 hours before either tissue culture inoculation or further storage at -70° C. When storage at -70° C was required it was for no longer than 72 hours. Urethral and endocervical specimens were cultured in 96-well microtiter plates with an im-

Negative cultures		Total cultured (n)
n	%	
6	54	11
14	54	26
9	50	18
0		2
0		2
31	52	60

munofluorescent technique used to identify inclusions, and a single passage was carried out from initial culture.¹² A similar procedure was followed for endometrial cultures, except that the specimens were inoculated onto monolayers growing on glass coverslips in vials and as many as three passages from the primary monolayer were carried out before the specimen was determined to be negative. After cultures had been obtained, all patients were treated with a 7-day course of tetracycline and single-dose antigonococcal therapy if required.

Statistical analysis was performed with Fisher's exact test (two-tailed).

Results

Twenty-nine of the 60 (48%) patients enrolled in the study had at least one positive culture for *C. trachomatis*. The positive sites and presenting diagnoses are given in Table I. Twenty-six patients (43%) had *C. trachomatis* isolated from the lower genital tract (endocervix, urethra, or both), while in three patients it was recovered only from the endometrium; in one, from the endometrium and urethra but not the endocervix; and in eight, from both the endometrium and endocervix. Thus 12 of 29 (41%) patients with *C. trachomatis* infection of the genital tract had positive endometrial cultures. The proportion of patients with positive cultures in either the upper or the lower tract was not influenced by the presenting diagnosis. However, it was influenced by the number of passages in tissue culture. Four of the 12 endometrium-positive cultures were positive in the initial monolayer, four after a single pass, one after a second pass, and three after a third pass.

Clinical and historical factors potentially associated with recovery of the organism are given in Table II. None of the patients with a positive culture had a prior history of clinical salpingitis, although five with positive endometrial cultures had had gonorrhea in the past. Women without a history of any sexually transmitted disease were more likely to have lower tract infection ($p = 0.005$) than women with such a history. This as-

sociation was not present for endometrial infection ($p = 0.75$). Four patients complained of pain in the preceding 4 to 14 days but had normal bimanual examinations. One had a positive endometrial and endocervical culture for *C. trachomatis*; one had gonorrhea with negative chlamydial cultures; one had a urethral culture positive only for *C. trachomatis* but also had mucopurulent cervicitis; and the fourth had an endometrial culture positive only for *C. trachomatis*.

Cervicitis correlated with the presence of *C. trachomatis* in the lower tract ($p = 0.01$) but not in the endometrium ($p = 0.08$). Mucopurulent cervicitis was associated only weakly ($p = 0.07$) with *C. trachomatis* isolation in the lower tract and not at all in the upper tract ($p = 0.26$). Overall, gonorrhea was present in 33 of 60 (55%) patients studied and did not correlate with presence or absence of either endometrial or lower tract chlamydial infection. It also did not correlate with the presence or absence of mucopurulent cervicitis ($p = 0.55$). Fourteen of 26 (54%) women with positive lower tract infection used oral contraceptives as opposed to nine of 34 (26%) culture-negative women ($p = 0.04$). A similar trend was observed for oral contraceptive use and endometrial infection, but it was not statistically significant ($p = 0.26$). The three women who had positive results for *C. trachomatis* in the endometrium only all had had bilateral tubal ligations.

Comment

The present study demonstrates that a substantial proportion of asymptomatic women at risk for infection with *C. trachomatis* not only have lower genital tract infection but also have upper tract infection. Duff et al.¹³ recovered more species of bacteria from endometrial samples obtained by brush biopsy through a triple lumen catheter than from those by transfundal aspiration. They did not cleanse the cervix before introducing the catheter, and the differences in numbers of different organisms recovered by these two methods were not statistically significant. However, their data do suggest that endometrial specimens can be contaminated by lower genital tract microorganisms even with the triple lumen catheter technique. Nevertheless, it is highly unlikely that many of the endometrial isolates recovered in the present study were introduced at the time of sampling. Four of the 12 women with positive endometrial cultures had negative endocervical cultures, and in another two the endocervical culture was positive only after passage, indicating low numbers of organisms. Also the geometric mean of the number of inclusion-forming units (infective chlamydial particles) recovered from the endocervix was the same for women with and without positive endometrial cultures (data not shown). One would expect that the more organisms present in the endocervix, the more likely con-

tamination would occur. Thus, while we cannot entirely exclude contamination of endometrial samples with the endocervical organisms, we conclude that most, if not all, of the isolates recovered from the endometrium were present at that site and were indicative of infection.

The natural history of chlamydial genital tract infection in women has not been fully defined. However, it has been estimated that approximately one third with untreated endocervical infection will develop clinical salpingitis.¹⁴ Some of the others will develop subclinical salpingitis resulting in sufficient tubal damage to cause infertility or ectopic pregnancy. The majority of women with tubal infertility and serologic⁴ or cultural^{15, 16} evidence of chlamydial infection do not give a history of prior clinical salpingitis. Thus the presumption must be that they have had chronic subclinical chlamydial salpingitis. Our observations are consistent with such a hypothesis. In addition they suggest that such spread may occur shortly after the acquisition of infection.

An association between chlamydial infection and oral contraceptive use was observed. This has been noted previously.¹ In addition women with no history of sexually transmitted diseases were more likely to be infected with chlamydia than women with a history of sexually transmitted diseases. This observation may reflect protective immunity resulting from prior chlamydial infections and will be presented more fully in a separate report (Katz BP, et al., submitted for publication). Three of the patients who had positive endometrial cultures did not have chlamydia recovered from the lower genital tract. Interestingly, all three had had bilateral tubal ligations while none of nine women with positive results at both upper and lower sites had had this procedure. Even though statistically significant, the biologic relevance of this association is not readily apparent.

Paavonen et al.¹⁰ performed endometrial aspirates and biopsies in 35 women, 34 of whom had objective evidence of mucopurulent cervicitis. Plasma cell endometritis was demonstrated in 14 (40%) of the women, and *C. trachomatis* was recovered from the endocervix in 86% of these, as opposed to 48% of women without histologic evidence of endometritis. In addition the majority of women with histologic evidence of endometritis also had clinical evidence of endometritis in the form of uterine tenderness and/or cervical motion tenderness. Chlamydia was recovered from 14% of endometrial aspirates from women both with and without endometritis.

In contrast, in the present study none of the patients had clinical evidence of endometritis or salpingitis, and only 15 (25%) were diagnosed as having mucopurulent cervicitis based on the appearance of the endocervical mucus. A larger number might have been so diagnosed if the additional criteria of >10 polymorphonuclear

leukocytes per 1000 $\times$ microscopic field of a Gram-stained smear of endocervical mucus been used.¹¹ Even so there was an association between the presence of cervicitis and recovery of *C. trachomatis* from the lower genital tract as previously reported.^{10, 11} However, by the criteria that we used, 54% of the women with lower genital tract infection and 50% of those with endometrial infection did not have cervicitis. Thus, while the presence of mucopurulent cervicitis may suggest a possible chlamydial infection, its absence does not exclude this diagnosis.

It is likely that the women we evaluated and those studied by Paavonen et al.¹⁰ reflect a continuum in a disease spectrum. Their patients may either have been infected for a longer period of time or have developed a more intense inflammatory response for other reasons. Had our patients been reevaluated without therapy at a later point in time, it quite possible that a higher proportion would have had cervicitis, as well as overt signs of endometritis and salpingitis.

The differences in recovery of *C. trachomatis* from the endometrium may reflect differences in the manner in which either the sample was obtained or the culture was performed. In the present study specimens were not considered negative until three blind passages to fresh monolayers had been performed, and two thirds of positive endometrial specimens were positive only after passage. It is also possible that as an inflammatory response is mounted the number of organisms in the endometrium available for recovery may decrease. In any case, whether the disorder was diagnosed by culture or by histologic results, both reports demonstrated a high incidence of upper tract infection.

In the present study 20% of women at risk for chlamydial infection and 41% of women with a chlamydial infection at any site had an endometrial infection. These data indicate that chlamydia infections are not confined primarily to the lower genital tract and that any woman at risk for a chlamydial infection is at risk for an upper genital tract infection with its attendant consequences. Such observations reinforce the need not only for diagnosis and treatment of high-risk individuals but also for the development of appropriate screening programs to detect infected individuals where the risk is somewhat lower, for instance, sexually active college women.

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A comparison between laser excisional conization and laser vaporization for the treatment of cervical intraepithelial neoplasia

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One hundred twenty patients with cervical intraepithelial neoplasia who were treated by laser excisional conization were compared with a group of 100 women who had similar diagnoses and were treated by laser vaporization. Every operation in the series was performed in the outpatient surgical unit or in the office. In two thirds of the cases the procedures were carried out with the patients under local anesthesia. The parameters studied included time required to perform the procedure, immediate and delayed bleeding, postlaser stenosis, healing time, patient discomfort, persistence or recurrence of disease, and risk of invasion. The results of this study revealed few disadvantages to performing a laser excisional procedure. Immediate and delayed complications were less frequent with laser excisional conization. The time required to perform an excisional conization was on the average only 4 minutes longer than that for a vaporization. Most significantly, the laser excisional cone provides a large and excellent specimen for the pathologist to review. (*AM J OBSTET GYNECOL* 1986;155:39-44.)

Key words: Cervical intraepithelial neoplasia, laser excisional conization, laser vaporization

A substantial number of papers have been published describing the technique and results of laser vaporiza-

tion for the treatment of cervical intraepithelial neoplasia.¹⁻³ Those surgeons having the greatest expertise with the carbon dioxide laser obtain comparable results with cure rates of >90%.⁴⁻⁶ A critique voiced by many gynecologists, relating not only to the use of the carbon dioxide laser but also to cryosurgery and electrocautery, has been the risk of inadvertently missing a focus of invasive carcinoma within the endocervical canal. Re-

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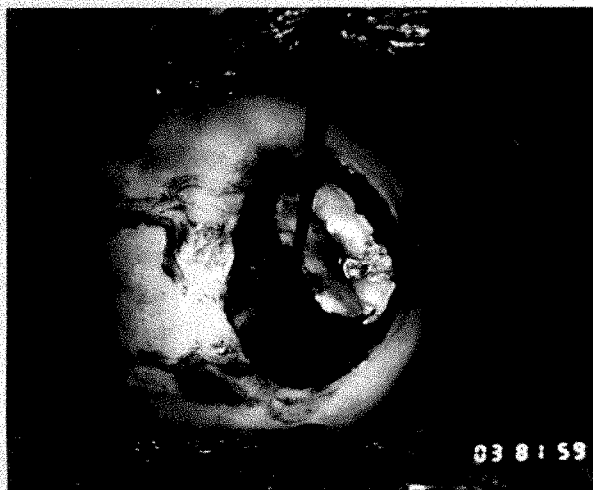


Fig. 1. A 10-inch titanium "laser" hook places traction on the cervical tissue specimen as the laser beam cuts deeply into the underlying stroma.

cently, this concern has heightened because of an increase in the number of endocervical adenocarcinomas being diagnosed.^{7,8} Townsend et al.⁹ have called attention to another problem associated with inappropriate, non-specimen-producing, end-stage therapy for intraepithelial disease, that is, oversights in the diagnostic workup resulting in mistaken diagnoses of cervical intraepithelial neoplasia where in fact invasive carcinoma exists. Inevitably, in any large series of conservative surgical procedures for cervical intraepithelial neoplasia, invasive carcinoma will occasionally be missed.

The technique of laser excision conization has been performed with increasing frequency since its original description.^{10,11} Recently, the technique of combining excision conization with vaporization has been described for the treatment of very extensive cervical intraepithelial neoplastic lesions.¹² Not only may laser excision conization be performed as an outpatient procedure, but the blood loss with the laser technique compared with that of conventional knife conization is substantially less.^{13,14}

This communication compares several parameters to determine the advantages versus disadvantages of carbon dioxide laser excisional conization and vaporization for the treatment of cervical intraepithelial neoplasia.

Material and methods

Between the years 1982 and 1985, 120 carbon dioxide laser excisional conizations were compared with 100 laser cervical vaporizations to determine the relative advantages and disadvantages of each procedure.

All women enrolled in this study had previous cervical cytologic testing, colposcopy, and colposcopically directed biopsies before the definitive laser surgical

procedures. Endocervical endoscopy and sampling of the endocervical canal were carried out when indicated. Endocervical cultures were obtained routinely for gonorrhea and chlamydia. Any patient having positive cultures was pretreated before the laser surgical procedure with tetracycline, 500 mg every 6 hours for 10 days. Additionally, beginning in 1984, every other patient with acute cervicitis shown on the biopsy, regardless of the culture report, was randomly treated with doxycycline, 100 mg every 12 hours for 10 days before the laser surgical procedure. Although indications for laser vaporization or excision were different, the laser excisional operation could be done in virtually every case, whereas the vaporization technique was limited to situations: (1) where the cervical intraepithelial neoplasia was located on the ectocervix only, (2) where there was no question of stromal invasion, (3) where no Papanicolaou smear-biopsy specimen disparity existed. Every laser procedure for the treatment of cervical intraepithelial neoplasia was performed in the outpatient surgical clinic with 68% of the cases being performed with the patients under local anesthesia. During the latter months of 1984 and the early months of 1985, 20 women underwent laser excisional conization in the office while under local anesthesia.

Laser operative procedures were performed with a Cooper-Niic 500-Z laser or a Sharplan Model 1100 laser. Both machines were capable of attaining powers of at least 50 W and spots ranging in size between 0.5 and 2 mm in diameter. Office conizations were performed with a Coherent Model 400 laser that obtained a maximum power of 22 W and a fixed spot diameter measuring 1.5 mm. Each laser was coupled to a Zeiss Opmi one microscope with a micromanipulator and a joystick used to direct the laser beam. Vaporizations were performed at 30 to 40 W of power with the use of a 1.5 mm spot and continuous wave with power densities varying between 1300 and 1800 W/cm². Laser excision conizations were performed at powers of 20 to 50 W with spots ranging between 0.5 and 1.5 mm in diameter and power densities between 888 and 20,000 W/cm².

The technique of vaporization has been previously described.⁴ Under colposcopic guidance the laser is used to outline the lesion with a 3 to 5 mm margin. Next, with the laser beam being moved rapidly, vaporization is carried down to a 5 to 7 mm depth. The endocervical margin is undercut circumferentially to a depth of 3 mm in order to produce eversion of the mucosa (buttoning).

The technique of laser excisional conization follows the general procedure described by Dorsey and Diggs,¹¹ but with *important modifications*. No lateral sutures are placed; 1 ml of vasopressin is mixed with 29 ml of 1% Xylocaine and about 10 ml of the solution is injected beneath the mucosa directly into the cervix. Three mil-

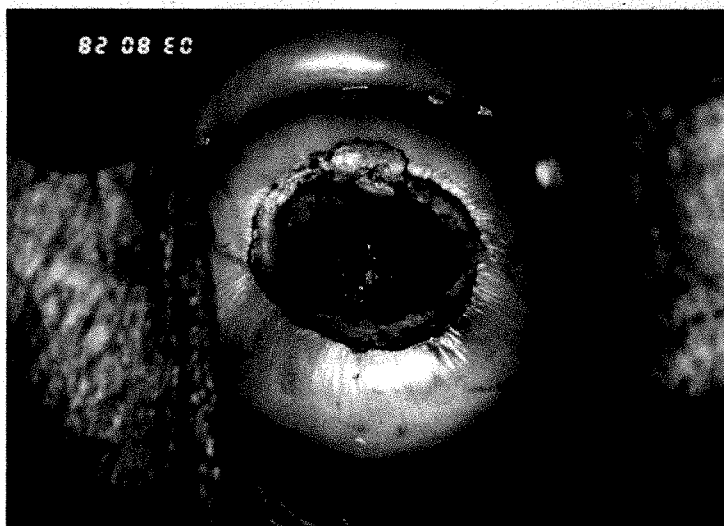


Fig. 2. The endocervical margin of the cone has been cut with a sharp scalpel in order to prevent any distortion. The cone bed is completely dry. The average time to complete this procedure is 12 minutes.

liliter syringes with 25-gauge needles are used to produce simultaneous anesthesia and vasoconstriction. A colposcopic examination is always performed before the injection of vasoconstrictor and the peripheral extent of the cone is outlined by imprinting a series of spots on the cervix. The laser power is turned up to 40 to 50 W and the spot size is reduced to 0.5 mm in diameter. An initial cut is made into the cervix, usually with penetration to a depth of 7 to 8 mm. A 10-inch titanium laser hook (Codman, Randolph, Massachusetts) is placed at a convenient site peripheral to the cone specimen and traction is placed on the cervical tissue (Fig. 1). The beam cuts deeply into the stroma as the operator angulates the beam toward the endocervical margin. As the hook is moved around the periphery of the specimen, the laser simultaneously cuts into the underlying stroma until the specimen is completely freed up, usually to a height of 1.5 to 2 cm. The endocervical margin is then cut with a sharp scalpel or the pulsed laser beam (300 pulses per second, 0.3 millisecond width) and the specimen is removed (Fig. 2).

The methods of anesthesia for both procedures are outlined in Table I. Local anesthesia is preferred and, as stated above, the anesthetic is injected directly into the cervix. This method of anesthesia renders the procedure completely painless for the patient. General inhalation anesthesia was administered only when specifically requested by the patient.

When intraoperative bleeding was encountered, laser power was cut to 10 to 15 W, and spots were enlarged to 2.0 mm in order to coagulate the bleeding vessel. When this was not successful, a 3-0 polyglycolic acid stitch was placed into the cervix to control bleeding.

Excisional cone sections were evaluated for clear

Table I. Anesthesia for outpatient treatment of cervical intraepithelial neoplasia

<i>Type of anesthetic</i>	<i>Excision conization</i>	<i>Vaporization</i>
General inhalation	45	22
Local injection into cervix	75	78

margins. Follow-up cytologic testing and colposcopy were performed initially at 12 weeks after treatment, then every 6 months. A patient was considered cured of disease when the Papanicolaou smear and colposcopic examination were normal for a period of 1 year or more. Only those women who underwent office conization have been followed up between 6 months and 1 year. The remainder of the patients were followed up between 1 and 3 years.

Results

Time. The average time required to vaporize a cervical lesion, with creation of a defect 2 cm in diameter, that is, the entire transformation zone, and 7 mm deep, was 8 minutes when no sutures were required to control bleeding. An average excisional cone measuring 2 by 1.5 to 2.0 cm required 12 minutes to complete. The mean time difference between the two procedures was 4 minutes. The average time required for the 20 excisional conizations performed in the office was 15 minutes.

Intraoperative and postoperative bleeding. Three patients undergoing laser vaporization had sufficient intraoperative hemorrhage to require the placement of a 3-0 vicryl suture to control the arterial bleeding. This

Table II. Results of carbon dioxide laser treatment in 200 cases of cervical intraepithelial neoplasia with minimum 1-year follow-up

Grade of cervical intraepithelial neoplasia	Laser excision conization		Laser vaporization	
	Cure*	Persistence/recurrence	Cure*	Persistence/recurrence
1	7	0	19	1
2	49	1	43	2
3	61†	2	32	3
Total	117	3	94	6

*Single laser treatment.

†One patient was diagnosed as having occult invasive epidermoid carcinoma and is not considered having a persistence or recurrence.

problem almost always occurred during vaporization of the posterior cervical quadrants and most often in cases of severe acute cervicitis in addition to cervical intraepithelial neoplasia (documented by the preoperative biopsy specimen).

Nine women had delayed bleeding that required examination to determine its source. Five of the nine women required some form of emergency treatment to stop the bleeding. Four of five had Monsel solution applied to an oozing bed. In one woman cervical suture was required to stop arterial bleeding.

No patient undergoing excision conization required placement of a suture because of acute bleeding. Three patients who had undergone laser excision conization had delayed postoperative bleeding occurring between 7 and 14 days postoperatively. Two of these were controlled with the application of Monsel solution and in one patient placement of a suture was required in the cervix.

None of the patients treated preoperatively with tetracycline or doxycycline had intraoperative or delayed bleeding requiring therapy. Regardless of whether postoperative bleeding occurred, the treated cervixes healed according to a previously published time sequence.¹⁵

Estimated blood loss. The average blood loss estimated intraoperatively for laser vaporization was 10 ml with a range of 0 to 200 ml. Of the three women who required intraoperative suturing, the blood loss was estimated at 150, 200, and 200 ml, respectively. No patient in this series required a blood transfusion.

The average blood loss for laser excisional conizations was also 10 ml with a range of 5 to 100 ml. Only one patient had an estimated blood loss of 100 ml. This was controlled by a combination of laser coagulation and the application of Monsel solution to the oozing bed of the conization site. Estimations were in fact semi-quantified since swabs used to clear the operative site were weighed before and after operation. By calculation of the differences between the wet and dry weights, the blood loss was measured.

Stenosis. Three of the 220 women (1.3%) developed

postoperative cervical stenosis. One patient had an extensive vaporization; one woman had an excisional conization, and one woman underwent a combination vaporization-excision procedure. The last patient underwent a subsequent repeat laser vaporization with exaggerated buttoning to remove the scar tissue, which corrected the stenosis.

Invasive carcinoma. One case of occult invasive epidermoid carcinoma was discovered in an excisional cone specimen. No patient undergoing vaporization of the cervix was diagnosed as having invasive cancer within the follow-up period.

Persistence or recurrence of cervical intraepithelial neoplasia. Table II shows the distribution and results of laser surgical procedures for cervical intraepithelial neoplasia. The overall cure rate for women undergoing vaporization (single treatment) was 94%. Persistence of disease, that is, disease present within the first 6 months of follow-up, was observed in five women. In such cases, neoplasia was always present at the endocervical or ectocervical treatment margin. One patient had recurrence or new disease at the 1-year follow-up examination. Interestingly, this woman who had been diagnosed as having grade 1 cervical intraepithelial neoplasia with condylomatous changes had extensive koilocytotic (warty) atypia extending over the ectocervical margin from 8 to 10 o'clock and at 4 o'clock.

Two women had cervical intraepithelial neoplasia present at the endocervical margin of the excised conization specimen. One additional patient was diagnosed as having invasive epidermoid carcinoma. The latter woman subsequently underwent radical hysterectomy for invasive disease. Extensive grade 1 cervical intraepithelial neoplasia with warty atypia was diagnosed at the 6-month follow-up visit in one patient.

No difficulty was encountered in establishing whether margins were clear after laser conization since the endocervical extremity was cut with either the knife or a superpulsed laser beam. A 3 mm margin at the ectocervical edge of the cone ensured accuracy of interpretation at this extremity.

Patient discomfort. There was no difference noted

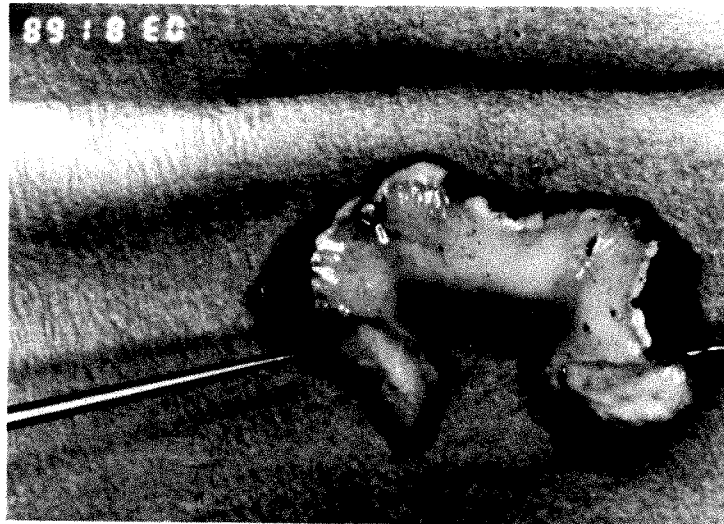


Fig. 3. The advantage of laser conization is the retrieval of a large specimen. The combination of very high-power densities, "fine hook" tissue manipulation, and no mucosal trauma ensures a fine specimen for the pathologist.

in the degree of postoperative discomfort between the two groups of patients. Approximately 7% of the women (both groups) complained of some cramping during the first 24 hours postoperatively. Motrin, 400 mg four times per day, proved to be the most effective treatment for relieving this problem. All patients were treated on a day-care basis and went home on the day of operation.

Comment

Laser operations for the treatment of cervical intraepithelial neoplasia offer the gynecologist a wide variety of methodologies and allow the surgeon to most appropriately tailor the operation to fit the geographic extent of the disease. This study showed few disadvantages to performing a laser excisional conization in preference to the technically simpler vaporization procedure.

Intraoperative and delayed postoperative bleeding happened more frequently with laser vaporization, three versus zero cases during operation and nine versus three after operation. Interestingly none of the patients who were pretreated with antibiotics developed bleeding as a result of either vaporization or excisional procedures. Although the mean estimated blood loss for both operations was equal, the vaporization group actually exhibited a higher range of blood loss. At least part of the advantage accrued to the excisional technique may, however, relate to the routine use of a vasoconstrictor before the laser procedure.

Postlaser stenosis was uncommon with one case each after vaporization, excision, and combination procedures. The risk of this complication could be diminished by performing the buttoning procedure, that is, evertting the endocervical margin with the laser. The

same technique could also be used for repairing stenotic cervixes.

The use of local anesthesia injected directly into the cervix resulted in complete pain relief in 75 of 120 women undergoing laser excisional conizations in this series. Direct cervical injection has consistently produced more reliable and more complete anesthesia than paracervical block in my hands. Effective local blockade and proper accessory instruments have allowed excisional conization to be performed safely and expeditiously in the office (20 cases). Since postoperative pain was minimal in both groups no additional analgesics were required.

Excisional conization, by providing extensive material for sampling, results in a copious specimen for the pathologist (Fig. 3). The quality of the laser cone is exceptionally good and the margins have presented no difficulty for pathologic interpretation. In this series, one case of invasive carcinoma of the cervix was discovered that might have been missed had the procedure been performed by vaporization. Although cure rates for cervical intraepithelial neoplasia are closely comparable, the excisional series showed a slight advantage, 97.5% versus 94%. This difference is more impressive if one isolates the results of treatment for grade 3 cervical intraepithelial neoplasia only. The cure rate for grade 3 cervical intraepithelial neoplasia by excision conization remained at 97% whereas that for vaporization dropped to 91%. Although the laser excisional conization is a technically more difficult operation to perform than laser vaporization, it is routinely taught to and mastered by new laser students. The operation also easily lends itself to outpatient and office settings.

Pure ectocervical lesions, regardless of extent or grade, may be effectively and easily eradicated by laser

vaporization. Lesions that extend into the endocervical canal should be treated by laser excisional conization. The advantage of a suitable specimen for the pathologist coupled with the minimal increase in time required to do the operation provides a sound basis for this recommendation.

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Prenatal diagnosis of craniofacial malformations with ultrasonography

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Although the utility of ultrasound in the prenatal diagnosis of many congenital anomalies is well established, its accuracy in detecting craniofacial malformations has not been examined in a large series. Sonographic examinations of 223 patients at risk for fetuses with craniofacial malformations were performed between 18 and 40 weeks. The risk factors included a familial history of craniofacial malformations, extrafacial anomalies diagnosed on ultrasound, fetal chromosomal aberrations, and maternal drug intake. Sonographic diagnosis was possible in 151 (67.7%) patients on the first scan and in 47 (21.1%) patients on the second scan and was not possible in 25 patients (11.2%). Of the 198 cases diagnosed antenatally, craniofacial malformation was detected in 14 and confirmed postnatally. No false positive diagnoses were made. A negative diagnosis of craniofacial malformation was made in 184 cases with two false negative results (1.0%). Anomalies diagnosed sonographically included anophthalmia, anterior cleft lip and/or palate, hypotelorism, hypertelorism, and micrognathia. The results of this study demonstrate that ultrasound is an accurate and reliable tool for the prenatal diagnosis of craniofacial malformations. (*AM J OBSTET GYNECOL* 1986;155:45-50.)

Key words: Craniofacial malformation, prenatal diagnosis, ultrasonography

Ultrasound is a well-established technique for the prenatal diagnosis of congenital anomalies arising from or involving the central nervous system, the heart, the visceral organs, and the skeletal system.¹

It is commonly recognized that craniofacial malformations may either arise from genetic transmission, chromosomal aberrations, or environmental insults or be a part of genetic or nongenetic syndromes with multiple organ involvement.^{2,3}

In recent years several case reports have demonstrated the sonographic diagnosis of craniofacial malformations.⁴⁻¹¹ However, the accuracy of ultrasound in detecting such anomalies has not been examined in a large series.

The purpose of this communication is to report a prospective study conducted in a group of patients with increased risk for fetuses with craniofacial malformations in whom ultrasound was used to evaluate the integrity of the craniofacial structures.

Patients and methods

Between 1980 and 1985, patients at risk for craniofacial malformations underwent ultrasound examina-

tions either at the Perinatal Unit of Yale University School of Medicine or at the Prenatal Pathophysiology Unit of the Bologna University School of Medicine. The risk factors considered in this study included familial history of craniofacial malformation, extrafacial anomalies detected by ultrasound, chromosomal aberrations detected by amniocentesis, and maternal intake of drugs associated with congenital craniofacial malformations.

A variety of ultrasound equipment was used for this study: General Electric Dataline, SRT L/S, RT 3000 (General Electric, Milwaukee, Wisconsin) and Technicare 280 SL (Johnson and Johnson, Independence, Ohio).

In all cases a midsagittal scan was first obtained to allow visualization of the fetal profile (Fig. 1); then a cross-sectional image of the fetal face was undertaken to assess the integrity of the nose and lips (Fig. 2). Axial scans were then used to evaluate the dimensions of the orbital diameters according to Mayden et al.¹¹ (Fig. 3) and to examine the upper lip and the anterior palate (Fig. 4). When the examination was considered unsatisfactory the patient was scheduled for a second evaluation.

Results

Two hundred twenty-three pregnancies were examined, with gestational ages ranging from 18 to 40 weeks. The indications for evaluation of the fetal face are reported in Table I. A satisfactory examination of the fetal face was obtained on the first scan in 151 cases

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Fig. 1. In this second-trimester fetus, a midsagittal scan of the face allows identification of the forehead, nose, mouth, and chin.



Fig. 2. A cross-sectional scan of the fetal face allows the visualization of the lens (*L*) inside the corpus vitreum, the eyelids (*E*), upper lip (*UL*) and inferior lip (*IL*), and nose (*N*).

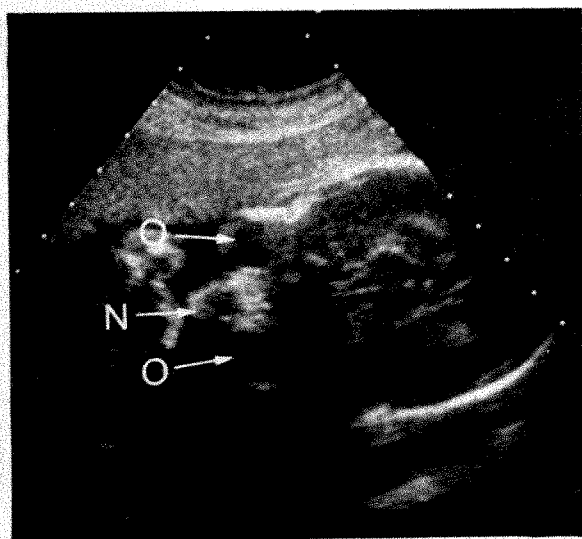


Fig. 3. Axial scan of the fetal head demonstrating the orbits (*O*) on both sides of the nasal bridge (*N*). This cross section can be used for the determination of the orbital measurements.

(67.7%) and at a second scan in 47 (21.1%). In 25 cases (11.2%), however, facial examination was not possible because of either an unfavorable position of the fetus (occipitoanterior, head low in the maternal pelvis) or associated conditions such as severe oligohydramnios and/or maternal obesity. Of the 25 cases in which ultrasound examination of the fetal face was not possible, there were two cases of craniofacial malformation diagnosed postnatally. The sonographic accuracy was assessed among the 198 patients in whom examination of the fetal face was possible.

Postnatal follow-up was available for all diagnosed cases. A diagnosis of craniofacial malformation was made in 14 cases and was confirmed on postnatal examination. In two cases, however, the diagnosis was only partially correct: In one fetus with holoprosencephaly, hypotelorism was recognized, but cleft lip and palate were missed. In one fetus with the Robin anomaly, a posterior cleft of the palate was not identified. There were, however, no false positive results (Table II). Detected anomalies included hypotelorism (Fig. 5),

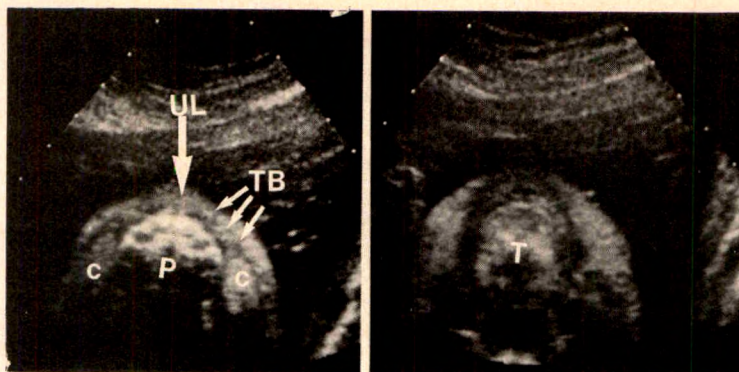


Fig. 4. Axial scans of the lower fetal face demonstrating the upper lip (UL), anterior palate (P), and, at a slightly lower level, the tongue (T) filling the oral cavity. At the level of the palate, the teeth buds (TB) can be appreciated, and the cheeks (C) are seen on both sides.

Table I. Indications for ultrasound examination of the fetal face

Indication	n	%
Other fetal anomalies detected by ultrasound	118	52.9
Familial history of craniofacial malformations	72	32.3
Maternal drug intake	25	11.2
Fetal chromosomal aberrations	8	3.6
Total	223	

arhinia, cleft lip and palate (Fig. 6), proboscis (Fig. 7), and micrognathia (Fig. 8).

Of the remaining 184 cases in which a diagnosis of no craniofacial malformation was made, there were two false negative results (1.0%). In one case, despite meticulous scanning on two different occasions, a posterior cleft of the palate was not identified. Another infant with cleft lip was born to a diabetic mother in whom ultrasound anatomic survey at 20 weeks was negative (Table III).

Comment

Our study demonstrates that ultrasound is feasible and reliable in the prenatal diagnosis of craniofacial malformations. A satisfactory examination of the fetal face was possible in the vast majority of cases. However, limitations in the evaluation of the fetal face resulted from an unfavorable position of the fetus, oligohydramnios, and/or maternal obesity.

Anomalies diagnosed sonographically included anophthalmia, anterior cleft palate and/or lip, hypotelorism, hypertelorism, and micrognathia. The posterior palate in the fetus is difficult to visualize sonographically, because of the acoustic shadowing that arises from the surrounding bony structures, and defects in this area will generally escape identification.

Micrognathia was demonstrated in two fetuses at 29 and 35 weeks of gestation; however, it is remarkable

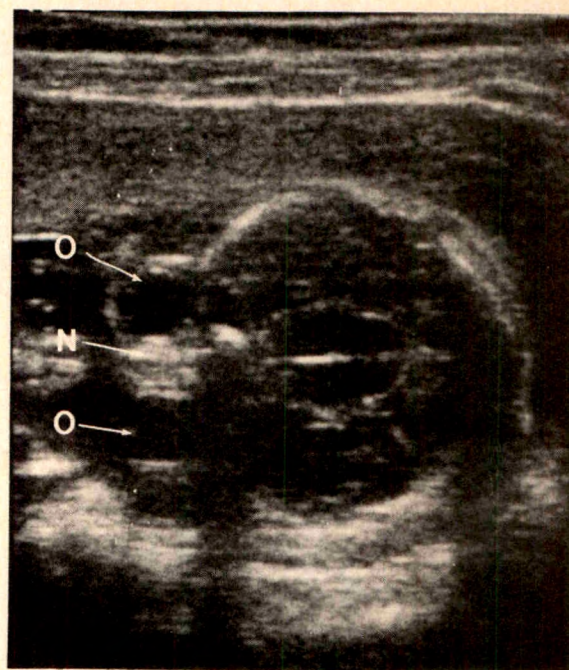


Fig. 5. Hypotelorism in a second-trimester fetus with holoprosencephaly and microcephaly (O = orbits, N = nose).

that in the latter case, ultrasound performed in the second trimester revealed a seemingly normal jaw. It is conceivable that in some cases micrognathia may evolve during the course of gestation, making an early diagnosis impossible.

No false positive diagnoses were formulated. Excluding one case of isolated cleft of the posterior palate, in only one case was a false negative diagnosis made.

Craniofacial malformations are among the most common congenital anomalies. The incidence of facial clefting has been reported to be as high as 1:600 births.^{3, 12} The observation that craniofacial malformations have genetic implications, being potentially transmitted through a polygenic or a monogenic pattern, creates the demand for prenatal diagnosis.^{2, 3}

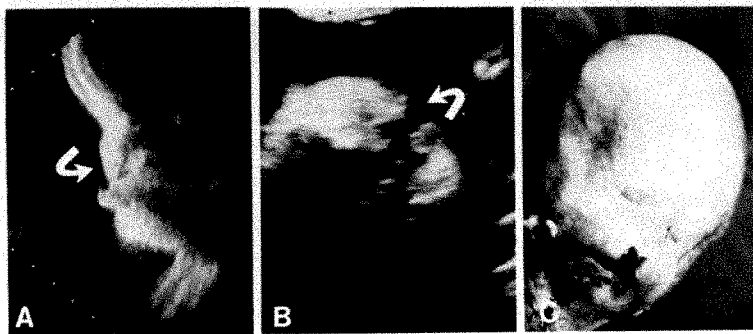


Fig. 6. Third-trimester fetus with holoprosencephaly. The profile view clearly demonstrates the absence of the nose (*A*), while the axial scan of the palate reveals a large median cleft (*B*). The appearance of the stillborn infant is shown in *C*.

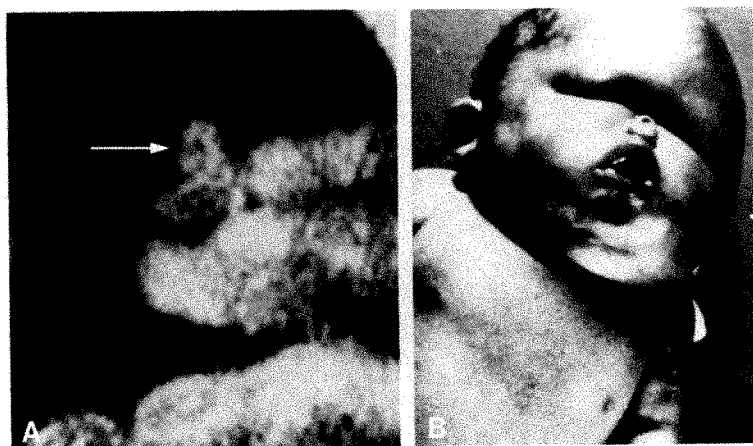


Fig. 7. Third-trimester fetus with holoprosencephaly and cebocephaly. The presence of a proboscis is sonographically demonstrated by the visualization of a single median opening (*arrow*) of the nasal appendage. The appearance of the newborn infant is shown for comparison.

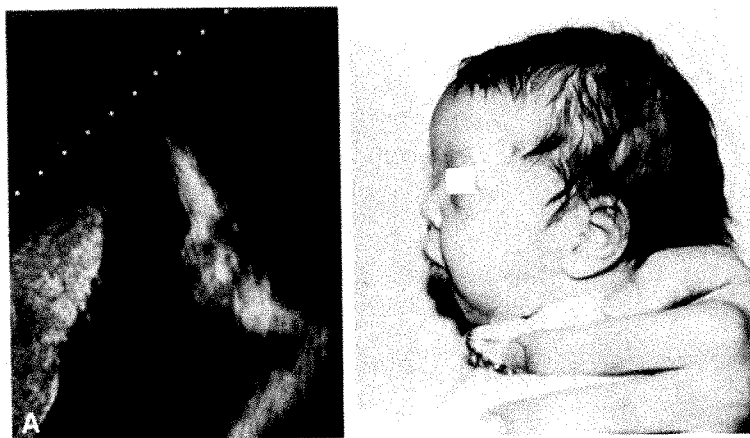


Fig. 8. The profile view of this third-trimester fetus reveals a strikingly small chin (*A*). The prenatal findings were confirmed at birth (*B*). (*B*, From Pilu G, Romero R, Reece EA, Jeanty P, Hobbins JC. The prenatal diagnosis of Robin anomalad. *AM J OBSTET GYNECOL* 1986;154:630.)

Our study indicates that the ability of ultrasound to exclude a craniofacial malformation is high. Such a capability should not be underestimated, as it allows the relief of parental anxiety.

Furthermore, craniofacial malformations could be

positively identified as early as the second trimester of pregnancy. Many craniofacial malformations, such as cleft lip and palate, can be treated after birth with good cosmetic results. It is expected that in these cases the prenatal recognition will not alter the obstetric man-

Table II. Craniofacial malformations diagnosed by ultrasonography

<i>Case No.</i>	<i>Indication</i>	<i>Sonographic findings</i>	<i>Weeks' gestation</i>	<i>Postnatal findings</i>
1-7	Fetal holoprosencephaly	Hypotelorism Median cleft lip and palate Absent nasal bridge	22-30	Holoprosencephaly Median cleft lip and palate Absent nasal bridge
8	Fetal holoprosencephaly	Anophthalmia Median cleft lip and palate Absent nasal bridge	29	Holoprosencephaly Anophthalmia Median cleft lip and palate Absent nasal bridge
9	Fetal holoprosencephaly	Hypotelorism Proboscis	30	Holoprosencephaly Hypotelorism Proboscis (cebocephaly)
10*	History of Robin anomalad	Micrognathia	35	Micrognathia Posterior cleft palate (Robin anomalad)
11	Anencephaly	Micrognathia	29	Anencephaly Micrognathia
12†	Hydrocephalus	Hypotelorism Median cleft lip	31	Hydrocephalus Hypertelorism Median cleft lip (median cleft face)
13	Fetal holoprosencephaly	Hypotelorism	28	Holoprosencephaly Hypotelorism Median cleft lip and palate
14	Microcephaly	Micrognathia	33	Microcephaly Micrognathia 47,XY +13

*Previously reported (see reference 6).

†Previously reported (see reference 12).

Table III. Craniofacial malformations not identified by ultrasonography

<i>Case No.</i>	<i>Indication</i>	<i>Sonographic findings</i>	<i>Weeks' gestation</i>	<i>Postnatal findings</i>
15	Maternal diabetes	Normal face	20	Cleft lip
16	Univentricular heart	Normal face	38	Posterior cleft palate
17	Holoprosencephaly	*	38	Holoprosencephaly Hypotelorism Median cleft lip and palate
18	Microcephaly	*	36	Holoprosencephaly Cyclopia

*face not accessible for ultrasound examination.

agement but will be helpful in removing the "surprise factor," allowing the parents to prepare psychologically for the birth of a child with a craniofacial malformation. In some cases, such as the Robin anomalad, a craniofacial malformation may jeopardize the newborn infant, and a planned delivery with immediate neonatal assistance can be lifesaving.⁴

Craniofacial malformations may be a part of a number of syndromes with multisystemic involvement and their recognition may have important clinical relevance. A classical example is represented by the holoprosencephalic malformations, in which anomalies of the brain and face are closely correlated.¹ The list of congenital syndromes that may be suspected or recognized by the detection of a craniofacial malformation is extensive and is beyond the scope of this report. Gorlin et al.,¹⁴ in an in depth review of the subject, indicate that approximately 150 syndromes involve craniofacial malformations with clinical implications.

Of the 18 cases of fetal craniofacial malformations

encountered in this study, polyhydramnios was found in 11 (61.1%). The increased amount of amniotic fluid could be related to a severe cerebral anomaly such as anencephaly or holoprosencephaly, possibly interfering with the control of fetal swallowing. However, in three cases (17.6%) no other anomalies were present. Since micrognathia and cleft palate by themselves can interfere with swallowing, we recommend that ultrasound examination of the fetal face become a part of the clinical evaluation of pregnancies complicated by polyhydramnios.

In the past, pregnancies at risk for craniofacial malformations were evaluated by fetoscopic examination.¹⁵ However, the results of our study indicate that fetal craniofacial malformation can be accurately detected or excluded in the early stages of pregnancy by ultrasonography, thus obviating the need for invasive procedures. The information provided not only may be useful to the parents but also may affect the obstetric and neonatal management.

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CA 125 antigen in human amniotic fluid and fetal membranes

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The cancer antigen CA 125 is manifest by serous cystadenocarcinoma of the ovary and to a lesser extent by other gynecologic and nongynecologic tumors. Its presence was screened for in normal human fetal tissues and fluids. Appreciable quantities of CA 125 were discovered in amniotic fluid by both a dot blot assay and the commercially available immunoradiometric assay kit. The most likely source of this antigen was found not to be the developing fetus, since antigen was absent from cord blood and fetal urine, but rather the chorionic membrane, which contained significant quantities of the antigen. CA 125 was found in extracts of maternal decidua, but none was found in extracts of placenta or amnion. The CA 125 antigen was determined by gel filtration experiments to be in excess of 700,000 daltons and probably in the range of 2 to 3×10^6 daltons. Size heterogeneity based on gel filtration and anion heterogeneity based on anion exchange chromatography have both been demonstrated for the CA 125 molecule. The amniotic fluid antigen is composed of two subunits of approximately 240,000 and 180,000 daltons as detected by iodine 125-labeled OC 125 monoclonal antibody. The antigen may contain additional subunits not detected by the monoclonal antibody. Size and charge heterogeneity as well as the poor definition of the subunit bands on polyacrylamide gels also suggest this molecule contains an appreciable carbohydrate component. (*AM J OBSTET GYNECOL* 1986;155:50-5.)

Key words: CA 125, human, amniotic fluid, amnion, chorion, decidua

The cancer antigen CA 125 has been established as a tumor marker for serous cystadenocarcinomas of the ovary.¹⁻⁴ The antigen was discovered by use of a monoclonal antibody screen of hybridomas after stimulation of mice with cells derived from an ovarian cancer cell

line, OVCA 433.⁵ The monoclonal antibody found to be most useful was named OC 125. The antigen specified by OC 125 is a glycoprotein of high molecular weight (over 200,000) that has been partially purified from tissue culture media.⁶ CA 125 antigen has been shown to be present in the sera of 85% to 90% of patients with ovarian carcinoma, 58% of patients with pancreatic carcinoma, 32% of patients with carcinoma of the lung, 22% of patients with carcinoma of the colon, and 30% of patients with miscellaneous gastrointestinal tract tumors.¹

Although rather extensive data have been collected

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that quantify CA 125 in sera from patients with malignant disease and from normal patients, little is known about the nature of the CA 125 antigen. Present data indicate the antigen is present on the cell surface of many cell lines of ovarian serous cystadenocarcinoma origin.⁶ It likely is sloughed or secreted from the surface of malignant ovarian cells, thus accounting for its presence in sera of patients with serous ovarian carcinoma. It generally is not detected in tissues of normal adults. However, when a biotin-avidin immunoperoxidase staining technique was used, trace amounts of CA 125 were identified in the fallopian tube, endometrium, and endocervix. As a result, it has been suggested that CA 125 is an antigenic determinant associated with müllerian differentiation. In addition, the antigen was identified by similar assay in fetal and adult serosal surface epithelium including pleura, peritoneum, pericardium, umbilical epithelium, and amnion.⁷ Recently Niloff et al.⁸ have described the presence of CA 125 in amniotic fluid.

The present investigation was undertaken to identify a readily available source of CA 125 so that its biochemical characteristics could be better described and factors controlling the expression of this antigen might be elucidated. In this report we characterize normal fetal tissue and fluids as a source of CA 125 in large quantity and describe its expression and distribution during normal development.

Material and methods

Tissues and specimens. Amniotic fluid obtained for amniocentesis was centrifuged; the fluid supernatant was collected and stored at -20°C until assayed. Cord blood, gastric aspirates, and fetal urine were obtained after normal delivery. Bloods were stored at -20°C as serum after clot removal. Fluids were stored directly at -20°C . Membranes, placenta, and decidual tissues were all obtained at the time of delivery, placed in ice-cold phosphate-buffered saline solution, and transported immediately to the laboratory. Blood clots were removed from tissues with several washes of cold phosphate-buffered saline solution. Amnion was dissected from chorion as a discrete sheet; the plane of cleavage was found routinely to be between the spongy layer of amnion and the cellular layer of chorion. These tissues were stored separately at -70°C .

CA 125 assay. All assays were carried out with use of a CA 125 assay kit purchased from Centocor Inc., Malvern, Pennsylvania, according to kit instructions. This immunoradiometric assay was first described by Klug et al.⁹

Exclusion chromatography. Initial preparation of amniotic fluid for chromatography was accomplished by filtering the fluid through a $1.25\text{ }\mu\text{m}$ filter followed by a fivefold concentration of the filtrate. Initially 10 ml of concentrate were applied to a $2 \times 100\text{ cm}$ column

containing HW 55 exclusion gel (fractogel HW 55 F) equilibrated with 20 mmol/L of bis-Tris-propane, pH 7.2, containing 0.15 mol/L of sodium chloride. The column was run at 0.5 ml/min and 5 ml fractions were collected. In subsequent experiments, 20 ml of sample was applied to a similarly prepared $5 \times 100\text{ cm}$ column containing HW 65 exclusion gel (fractogel HW 65 F) and 9 ml fractions were collected.

Fast protein liquid chromatography. Fractionation of amniotic fluid was accomplished by applying a 500 μl sample to a mono Q anion exchange column (Pharmacia) and eluting the sample with a linear gradient of 0.015 mol/L of sodium chloride to 1.5 mol/L of sodium chloride containing 20 mmol/L of bis-Tris-propane, pH 7.2. The gradient control and the flow rate (1 ml/min) were effected by an LCC automated chromatography control unit (Pharmacia); 1 ml fractions were collected.

Tissue extraction. Tissues stored at -70°C were minced, after thawing, in 4 ml of phosphate-buffered saline solution per gram of tissue. The minced tissue was then homogenized with use of 5 by 5 second bursts on a Tekmar Tissuemiser. The homogenate was centrifuged at $10,000 \times g$ for 20 minutes at 4°C and the supernatant assayed for CA 125.

Dot blots analysis. Dot blotting was carried out by transferring 150 μl aliquots of fractions collected from anion exchange chromatography to nitrocellulose filters under low vacuum in a micro sample filtration manifold (Schleicher & Schuell Inc.). Nitrocellulose filters were then washed with 10 ml of blotto (5% weight per volume of milk powder in phosphate-buffered saline solution) by shaking in a sealed plastic bag for 30 minutes. Washed filters were then exposed to ^{125}I -labeled OC 125 (the monoclonal antibody for CA 125 labeled with radioactive ^{125}I) at a concentration of 10^6 cpm per 10 ml overnight. Nonspecific binding was reduced by washing the filters three times in 10 ml aliquots of blotto. Filters were exposed to x-ray film at -70°C for 2 days and developed in an automatic x-ray developer unit.

Sodium dodecyl sulfate polyacrylamide gels. Gradient polyacrylamide gels (9% to 15%) were run according to Laemmli.¹⁰ Protein preparations were first reduced and dispersed by incubation in sample buffer (60 mmol/L of Tris, pH 6.8, 0.05% mercaptoethanol, 3.0% sodium dodecyl sulfate) at 100°C for 2 minutes; 25 μl aliquots were then electrophoresed for 4 hours at 30 mA. After electrophoresis the polyacrylamide gel was equilibrated with Tris glycine transfer buffer (25 mmol/L of Tris, pH 8.3, 192 mmol/L of glycine) and transferred to nitrocellulose filters by means of a transblot cell (Biorad Inc) in the presence of 20% methanol at 30 Volts overnight. Filters were then exposed to ^{125}I antibody OC 125 in blotto as described for dot blots.

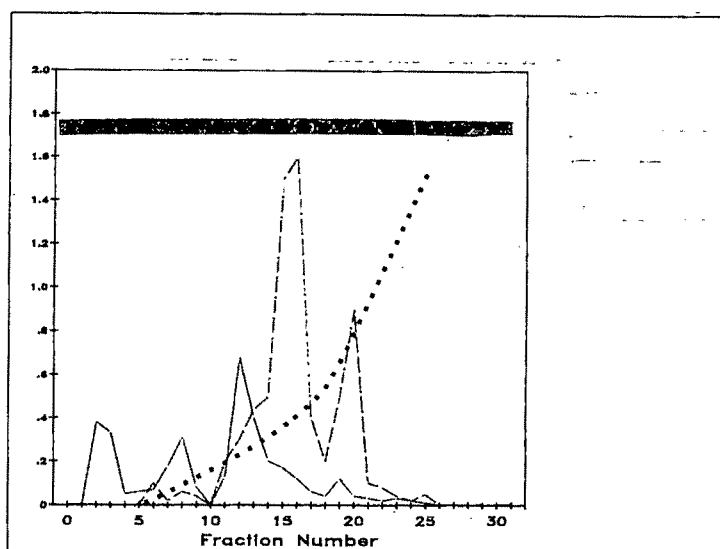


Fig. 1. Fast protein liquid (FPLC) anion exchange chromatography of amniotic fluid. Five hundred microliters of amniotic fluid was filtered through a 1.25 μ m millipore filter before chromatography at Ph 7.2 on a mono Q anion exchanged column (Mono Q, FPLC, Pharmacia Inc.) with use of a sodium chloride gradient (■) from 0.015 to 1.5 mol/L. One milliliter fractions were collected and assayed for CA 125 (●, cpm $\times 10^{-3}$) using a monoclonal immunoradiometric assay produced by Centocor Inc., Pennsylvania, or 150 μ l was dot blotted to nitrocellulose filters, incubated with ^{125} I-labeled OC 125 antibody, and exposed to x-ray film. —, Optical density.

Table I. CA 125 in amniotic fluid

Specimen No.	Gestational age (wk)	CA 125 (U/ml)
1	13	7,375
2	15	7,450
3	15	9,875
4	15.5	4,750
5	16	4,500
6	16	7,450
7	16.5	13,000
8	16.5	7,200
9	17	3,250
10	17	5,250
11	18	7,750
12	18	4,450
13	19	9,500
14	20	6,050
15	26	5,750

Results

Human amniotic fluid obtained throughout pregnancy has proved to be a rich source of CA 125 antigen. When amniotic fluid was filtered and then chromatographed on an anion exchange column with use of a sodium chloride gradient (0.015 to 1.5 mol/L), both the immunoradiometric assay system and a Western dot blot assay identified the presence of CA 125 in the same fractions (Fig. 1). Levels of CA 125 of 800 to 1000 U/ml have been observed by Kabawat et al.⁴ in sera from patients with extensive tumor burden. Amniotic fluid from normal gestations has now been shown to have even higher CA 125 concentration (Table I).

Table II. Amniotic fluid CA 125 in twin pregnancies

Twin pregnancy No.	Twin infant	Gestational age (wk)	CA 125 U/ml
1	A	17	5,875
	B		6,870
2	A	26	10,000
	B		11,000
3	A	35	7,250
	B		7,000
4	A	Term	2,000
	B		1,900

Although a large number of amniotic fluid samples have not been assayed thus far, we have found no striking relationship between CA 125 concentrations and length of gestation. A wide range in CA 125 concentrations seems to be typical. Such also seems to be the case in twin gestations. In four pairs of amniotic fluid specimens from twin pregnancies, no differences in CA 125 concentrations were found between twins A and B (Table II) even though a wide range of concentration of CA 125 was observed among individual twin pregnancies.

We next sought to identify the source and distribution of CA 125 in human pregnancy. Serum obtained from normal pregnant women at various gestational ages (range, 10 to 40 weeks) had an average concentration of CA 125 of only 30 U/ml. Because significant

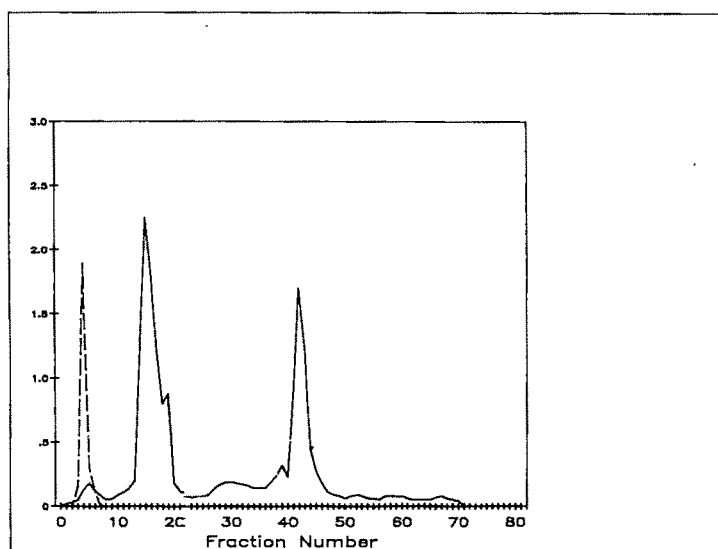


Fig. 2. Fractogel HW 55 F (E. M. Reagents) gel filtration chromatography of amniotic fluid. Fifty milliliters of amniotic fluid were concentrated fivefold in dialysis tubing with use of Aquaside II (Calbiochem). A fluid volume of 10 ml was layered over a 2×100 cm column of HW 55 F (fractionation range 10,000 to 700,000 daltons) and chromatographed at a flow rate of 0.5 ml/min. Five milliliter fractions were collected and assayed for CA 125 activity (---, cpm $\times 10^{-3}$). —, Optical density.

Table III. CA 125 in maternal and fetal body fluids

Fluid	No. of samples	CA 125 U/ml
Cord serum	6	17
Fetal urine	4	25
Gastric aspirate	6	4510
Maternal serum	9	28

quantities of CA 125 were present in amniotic fluid at term, we measured concentrations of the antigen in cord blood in first-voided neonatal urine specimens, and in gastric aspirates of newborn infants in a preliminary attempt to identify the source of this CA 125. Neither cord blood nor first neonatal urine had significant concentrations of CA 125 (Table III). In contrast, gastric aspirates contained significant concentrations of CA 125, but these levels did not differ from levels found in amniotic fluid and therefore were probably derived from amniotic fluid.

To evaluate further the source of CA 125 in amniotic fluid, CA 125 was assayed in tissue extracts of fetal membranes, placenta, and maternal decidua vera from women undergoing elective cesarean delivery. The amniotic membranes were totally separated from the chorion and divided into two categories (1) membrane overlying the placenta (placental amnion and placental chorion) and (2) membrane surrounding the remainder of the gestational sac (extraplacental amnion and extraplacental chorion). Compared to amnion and pla-

Table IV. CA 125 in trophoblast and maternal tissues

	CA 125 (U/gm of tissue)
Placental amnion	255
Extra placental amnion	330
Placental chorion	41,749
Extra placental chorion	44,930
Placenta	248
Decidua	29,411

centa, there were extraordinary quantities of CA 125 in the $10,000 \times g$ supernatant of extracted chorionic membrane. No "geographic" differences in the distribution of CA 125 within chorion were observed; that is to say, extracts of chorion overlying the gestational sac and extracts of chorion overlying placenta had similar concentrations of antigen in their $10,000 \times g$ fractions (Table IV). Significant quantities of CA 125 were recovered for the $10,000 \times g$ supernatant fraction of decidua. It is yet to be determined whether this represents a synthetic source of CA 125 or whether this represents contamination from the overlying chorion.

CA 125 eluted in the void volume of a gel matrix designed to fractionate molecules up to 700,000 daltons, indicating that CA 125 is totally excluded from the matrix of that column (Fig. 2). Further, chromatography of antigenic activity over a second exclusion matrix that is designed to separate molecules in the range of 5 million to 50,000 daltons indicated that this

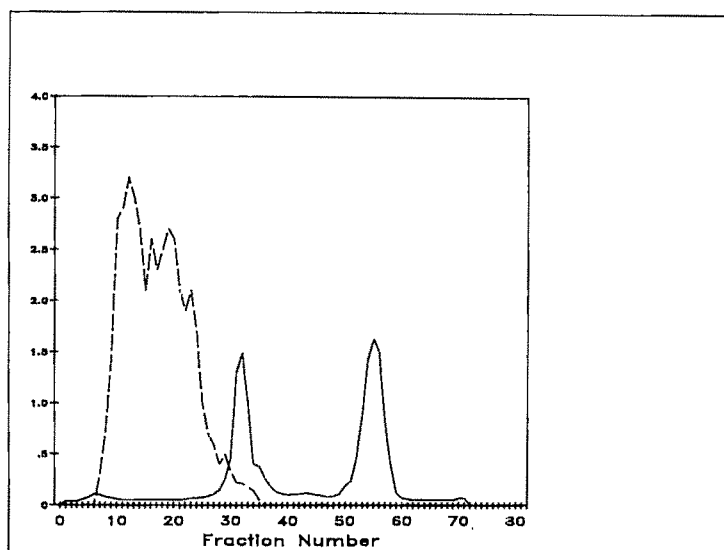


Fig. 3. Fractogel HW 65 F (E. M. Reagents) gel filtration chromatography of amniotic fluid. Amniotic fluid was prepared as described in Fig. 2. Twenty milliliters of $5\times$ concentrated amniotic fluid were applied to a 5×100 cm column of HW 65 F (fractionation range 50,000 to 5,000,000 daltons). Nine milliliter fractions were collected and assayed for CA 125 activity (---, $\text{cpm} \times 10^{-3}$). —, Optical density.

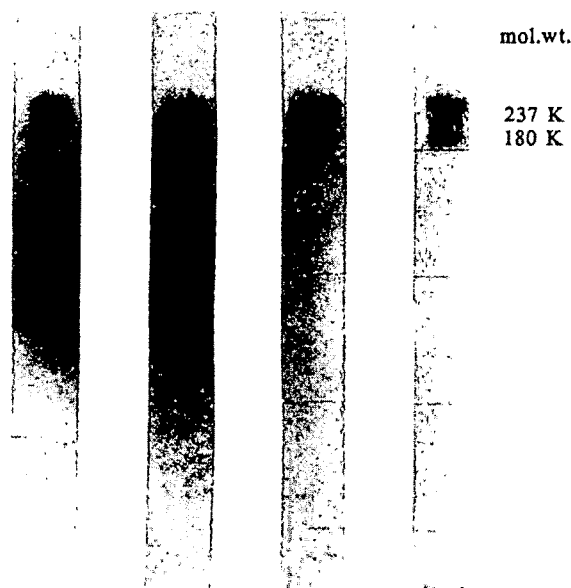


Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of amniotic fluid CA 125. Twenty-five microliter aliquots of amniotic fluid were electrophoresed over a 9% to 15% polyacrylamide gradient gel after dispersion in sodium dodecyl sulfate and mercaptoethanol. After electrophoresis, proteins were transblotted electrophoretically to a nitrocellulose filter, and the filter was probed with ^{125}I -labeled OC 125 antibody. Bound radioactivity was determined by exposing the filters to x-ray film. Molecular weights were determined by cochromatography of molecular weight standards after removal of the gel lanes containing the standards, followed by Coomassie blue staining.

antigen coeluted in the range of blue dextran and showed considerable size heterogeneity (Fig. 3). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of CA 125 followed by transblotting to nitrocellulose and probing with ^{125}I -OC 125 antibody indicated two major bands of antigenic binding at approximately 186,000 and 237,000 daltons (Fig. 4). It was further noted that on several occasions these bands were more diffuse or less discrete.

Comment

A significant concentration of the CA 125 antigen has been found in amniotic fluid, which is equivalent to the highest concentrations found in the sera of patients with a large tumor burden. The most likely source of this antigen is suspected to be the chorion, since very little CA 125 was detected in fetal serum or urine. Significant concentrations of antigen were discovered in decidual tissues, but little or no antigen was discovered in amnion or placental extracts. Levels of antigen were highest in extracts of chorion and as such the possibility of decidual contamination with CA 125 from chorion is possible. The nature of the CA 125 molecule is unusual in several ways. As indicated in Fig. 2, CA 125 is an extraordinarily big molecule, at least as indicated by molecular sieving chromatography in which profiles suggest molecular size as high as 2 to 3×10^6 daltons with notable size heterogeneity (Fig. 3). Both the size and the molecular heterogeneity point to a glycoprotein structure with major polysaccharide content. Subunit

analysis of CA 125 on sodium dodecyl sulfate reducing gels indicated the presence of two subunits (237,000 and 186,000 daltons). Because both subunits are detected by a monoclonal antibody (OC 125) these data indicate the presence of shared antigenetic determinants but do not preclude the presence of other subunits not recognized by the monoclonal antibody. It is also possible that the lower molecular weight subunit (186,000 daltons) is a breakdown product of the 237,000 molecular weight species and therefore contains the same antigenic determinant. The diffuse pattern of the bands detected by the monoclonal antibody is typical for glycopeptides and would further implicate a carbohydrate nature to the CA 125 molecule. The detection and partial characterization of the CA 125 molecule synthesized during normal fetal development by trophoblast-derived chorionic cells provides a foundation for an evaluation of the expression of this gene in normal and transformed tissues. The detailed structure, function, and expression of the CA 125 molecule in normal gestation is presently under investigation.

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The CA 125 assay as a predictor of clinical recurrence in epithelial ovarian cancer

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Serum CA 125 levels were obtained from 55 women with epithelial ovarian cancer before a second-look surgical procedure and serially thereafter. All patients were clinically and radiographically free of tumor at the time of the second-look operation and were followed to clinical recurrence. Median follow-up was 12 months. CA 125 levels obtained at the second-look operation had a sensitivity and specificity for predicting clinical recurrence of 94% and 88%, respectively. Patients with an elevated CA 125 level (≥ 35 U/ml) had a 60% chance of clinical recurrence within 4 months, while patients with levels < 35 U/ml had a 5% chance of clinical recurrence over the same time period. Serial CA 125 levels obtained after second-look operations were strong predictors of clinical outcome, and distinctly different monitoring profiles were observed among those patients remaining clinically free of tumor and those suffering clinical recurrence. The CA 125 assay became elevated (≥ 35 U/ml) before clinical recurrence in 94% of 35 cases with a median lead time of 3 months. The CA 125 assay identifies patients destined to suffer a clinical recurrence and provides a warning measurable in months. This may have important implications for therapy. (AM J OBSTET GYNECOL 1986;155:56-60.)

Key words: CA 125 radiometric assay, ovarian cancer, second-look surgical procedure

Ovarian cancer spreads in an occult intraperitoneal fashion and often remains confined to the peritoneal cavity for a prolonged period of time late into the course of the disease. It is thus difficult to detect clinically by palpation.^{1,2} The use of second-look and sometimes multiple laparotomies is therefore widely used to assess intraperitoneal tumor status among patients with epithelial ovarian cancer.¹⁻³ The CA 125 radiometric assay when elevated at the time of a second-look surgical procedure indicates the presence of intraperitoneal tumor.^{6,7} However, the presence or absence of tumor may not be the most important parameter. Patients with persistent tumor at a second-look operation may have a prolonged survival. In one report, which was based on 50 patients with microscopic disease at second-look laparotomy, the 2- and 5-year survival rates

were 96% and 71%, respectively.⁵ Fifty-three percent of Wharton et al.'s 96 patients who survived over 48 months had tumor present at the second-look operation and several had macroscopic disease.⁸ Long-term survivors have been reported by others following positive second-look procedures,⁴ including one report of seven patients with survivals in excess of 5 years.¹ Survival is the most important parameter and is closely associated with clinical recurrence. It would therefore be useful to have a tumor marker that could predict clinical recurrence. Inasmuch as prolonged chemotherapy may be associated with life-threatening complications,⁸ such a tumor marker might aid the physician in selecting appropriate patients and the appropriate timing for the institution of second-line chemotherapy. The goal of this study was to determine whether serum CA 125 levels obtained either at second-look operation or serially thereafter among patients clinically free of tumor could predict clinical recurrence and provide significant lead time. Analysis was also performed to determine the prognostic lifetime of a negative CA 125 assay.

Methods

Fifty-five patients at four institutions (Brigham and Women's Hospital, Hershey Medical Center, Mayo Clinic, and UCLA Medical Center) with epithelial ovarian carcinoma were followed with serum CA 125 levels after undergoing a second-look operation. The CA 125

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Table I. Patient population

	<i>n</i>	%
No. of patients	55	
Median age (yr)	54	
Range (yr)	37-74	
FIGO stage		
I	1	2
II	3	3
III	44	80
IV	7	7
Histologic findings		
Serous	34	62
Endometrioid	6	11
Mucinous	1	2
Clear cell	1	2
Mixed	3	5
Unclassified	10	18
Tumor grade		
Borderline	1	2
1	2	3
2	8	15
3	41	75
4	2	3
Unknown	1	2
Residual disease following initial cytoreductive operation		
No residual disease	9	16
Tumor <1.5 cm	34	62
Tumor ≥1.5 cm	7	13
Unknown	5	9

assays were performed at a median interval of 3 months. All patients were clinically and radiographically free of tumor at the time of their second-look procedures. The timing of a subsequent operation, if performed, and the choice of procedure (laparotomy or laparoscopy) was left to the discretion of the physician. Patients were followed until clinical recurrence according to protocols in use at each institution. Recurrence was defined as palpable or radiologic evidence of tumor. The median follow-up was 12 months. The results were analyzed to determine whether CA 125 levels predict clinical recurrence.

Patients. The selection criteria of the 55 women with epithelial ovarian carcinoma included in this study have been described.⁷ Patient characteristics are described in Table I. All patients were initially treated with combination chemotherapy; 85% of the regimens included cisplatin. Each patient was clinically and radiographically free of tumor at the time of the second-look operation (29 laparotomies, 26 laparoscopies). Tumor was detected at operation in 36 individuals and was absent in 19. The CA 125 levels and surgical findings associated with these procedures have been described in detail.⁷ During the post-second-look study period 6% received additional chemotherapy, 26% immunotherapy, 16% radiation therapy, and 52% no further therapy. Thirty-five percent of the patients had third-look surgical procedures, 9% fourth-look, 5% fifth-look, and 2% sixth-look. These 28 subsequent procedures were

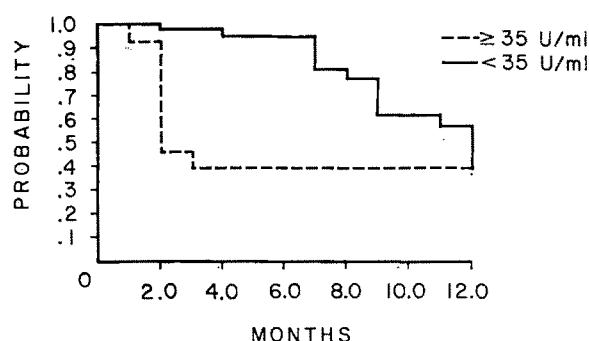


Fig. 1. Life table of time to clinical recurrence for patients with and without elevated CA 125 levels (≥ 35 U/ml) at second-look surgical procedures.

Table II. Correlation of the CA 125 level at the time of the last surgical evaluation and subsequent clinical outcome*

CA 125 at last surgical evaluation	Clinical outcome	
	Clinically free of tumor	Clinical recurrence
≥ 35 U/ml	2†	31
< 35 U/ml	15	2

*Five patients excluded because CA 125 levels were not available from time of operation.

†Tumors in these two patients have subsequently recurred.

equally divided between laparotomies and laparoscopies. Over the median follow-up period of 12 months after second-look operation 35 patients recurred and 20 remained clinically free of tumor.

CA 125 assays. Serum assays were performed in duplicate⁹ with use of kits provided by Centocor, Inc. (Malvern, Pennsylvania). CA 125 present in 100 μ l aliquots of serum is bound by purified OC 125 on a solid-phase immunoadsorbent. As more than one CA 125 site is associated with each antigen molecule, bound antigen is detected by incubation with iodine 125-labeled OC 125. CA 125 is expressed in arbitrary units based on comparison with a primary reference standard. Based on prior studies a CA 125 level ≥ 35 U/ml was defined as elevated.^{9,10}

CA 125 levels remained coded until the completion of the study and therefore did not influence clinical management. A clinical review of the patient data was performed by one investigator (R. C. K.) with no knowledge of the CA 125 values.

Statistical analysis. The significance of relationships between CA 125 levels and tumor recurrence status was calculated with use of Fisher's exact test.¹¹ Life tables for the time to clinical recurrence were constructed with use of the Kaplan-Meier product limit procedure.¹² The log rank test¹³ was used to compare the life table curves,

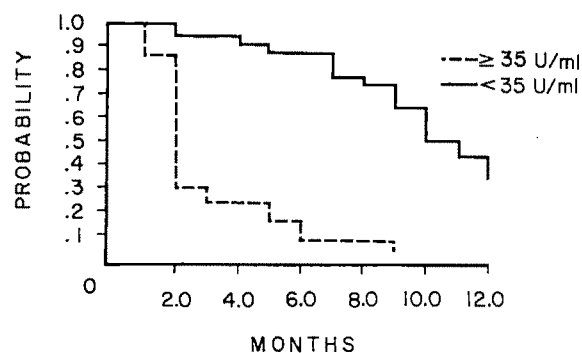


Fig. 2. Life table of time to clinical recurrence based on CA 125 levels at the time of second-look or subsequent-look surgical procedures.

while the Cox proportional hazards model¹⁴ was used to control for covariates in testing the value of elevated assays.

Results

CA 125 levels at second-look surgical procedures.

The sensitivity and specificity of the CA 125 level obtained just before surveillance operation for predicting clinical recurrence was calculated. The last surgical procedure (second or subsequent look) was used in this analysis to ensure a fair evaluation. This provided an unbiased sensitivity estimate but undoubtedly underestimated the specificity if patients clinically free of tumor later developed recurrence. As illustrated in Table II, the relationship between CA 125 levels at the last surgical evaluation and subsequent clinical outcome was highly significant ($p < 0.0000001$, Fisher's exact test). The sensitivity and specificity were 94% and 88%, respectively.

Time to clinical recurrence after second-look surgical procedures. The time to clinical recurrence after second-look operation was compared for patients with and without elevated CA 125 levels with use of a life table analysis (Fig. 1). Patients with assays of ≥ 35 U/ml at the time of second-look operation had significantly shorter times to clinical recurrence ($p < 0.001$, log rank test) than patients with assays of < 35 U/ml. An elevated CA 125 level at second-look operation was associated with a 60% chance of clinical recurrence within 4 months compared with 5% for levels of < 35 U/ml. For those patients with CA 125 levels exceeding 35 U/ml at a second-look operation, the greatest risk for recurrence was between 2 and 4 months following the procedure. These CA 125 predictions were independent of the degree of initial cytoreductive operation, which was also a prognostic factor ($p < 0.01$, Cox proportional hazard model) for clinically disease-free interval (Table III). If a similar life table is constructed instead with CA 125 levels obtained at the time of the last surgical evaluation (third or fourth look, etc.), the

Table III. Relationship between CA 125 levels at second-look procedures, completeness of initial cytoreductive operation, and clinical outcome*

Completeness of initial cytoreductive surgery	Percentage with clinical recurrence			
	CA 125 <35 U/ml		CA 125 ≥ 35 U/ml	
	%	n	%	n
No residual disease	0	0/8	0	0/1
Tumor <1.5 cm	62	13/21	100	8/8
Tumor ≥ 1.5 cm	100	3/3	75	3/4
Total	50	16/32	85	11/13

*Five patients excluded because of unknown extent of initial operation and five patients excluded because CA 125 levels were not available from time of second-look operation.

differences in time to clinical recurrence are even more pronounced (Fig. 2).

Examination of the life table (Fig. 1) indicates the lifetime of the prognostic value of a CA 125 assay performed at the time of a second-look operation. The subgroup with elevated CA 125 levels are at greatest risk for recurrence during the first 4 months, while those with second-look levels of < 35 U/ml are at increasing risk of clinical recurrence after the first 4 months. However, the probability of clinical recurrence is 60% for both subgroups after 1 year, which suggests that the prognostic value of the CA 125 assay does not exceed 1 year.

CA 125 levels after second-look surgical procedures. The relationship between the highest CA 125 level obtained after second-look operation and subsequent clinical outcome is illustrated in Table IV. The chance of observing this relationship if CA 125 levels were independent of the risk for clinical recurrence is less than one in a million (Fisher's exact test). Thus monitoring assay levels after second-look operations were strongly correlated with clinical recurrence and, as illustrated in Table IV, were of most value in the subgroup of patients with residual disease after the initial cytoreductive operation. Only 20% of those with CA 125 levels always < 35 U/ml recurred, while 93% of those with CA 125 levels > 35 U/ml eventually recurred. For all 55 patients the sensitivity and specificity of CA 125 levels for detecting clinical recurrence was 94% and 90%, respectively. The predictive values of a positive and negative CA 125 level were also 94% and 90%, respectively.

CA 125 monitoring profiles. Characteristic CA 125 trends were observed among patient subgroups. Those who were destined to suffer a clinical recurrence typically had progressively rising CA 125 levels. Three patients had sustained levels always > 35 U/ml. There

Table IV. Relationship between highest CA 125 assay after second-look procedures, completeness of initial cytoreductive operation, and clinical outcome*

Completeness of initial cytoreductive operation	Patients with clinical recurrence			
	Highest CA 125 assay <35 U/ml		Highest CA 125 assay ≥35 U/ml	
	%	n	%	n
No residual disease	0	0/8	0	0/1
Tumor <1.5 cm	22	2/9	96	24/25
Tumor >1.5 cm	0	0/1	83	5/6
Total	11	2/18	91	29/32

*Five patients excluded because of unknown extent of initial operation.

were only two false negatives in the clinical recurrence group. Among those who remained clinically disease free, the most frequent profile was a gradual reduction in CA 125 levels over time. Two of 20 patients had occasional CA 125 levels exceeding 35 U/ml in the absence of clinical recurrence and are described in detail below.

Lead time. Thirty-five patients had a clinical recurrence after a second-look operation. Serial CA 125 levels rose to ≥35 U/ml in 94% of these patients before their recurrences became clinically detectable. The median lead time before clinical recurrence was 3 months, and in over 90% of patients was at least 2 months (Fig. 3). Only two subjects had no lead time. Thirty-three percent of patients had lead times in excess of 6 months.

Among the 20 patients who did not recur clinically, CA 125 levels were always <35 U/ml in 18 (90%). One of the remaining two patients had a single elevated level (180 U/ml) 6 days after a negative second-look procedure and all subsequent assays were <35 U/ml. The other patient had serial CA 125 levels ranging between 11 and 55 U/ml following a negative second-look operation.

Comment

CA 125 is an antigenic determinant associated with a glycoprotein of high molecular weight. This determinant is recognized by the murine monoclonal antibody OC 125. With use of a radiometric assay developed to detect CA 125 in serum,¹⁰ elevated (>35 U/ml) antigen levels were observed in over 80% of patients with ovarian cancer but in only 1% of apparently healthy individuals.⁹ Serial levels correlated with disease progression or regression in 93% of instances, which suggests that this serum assay is useful for monitoring disease status in patients with epithelial ovarian cancer.⁹ Subsequent studies have demonstrated that CA 125 levels may predict intraperitoneal tumor status at

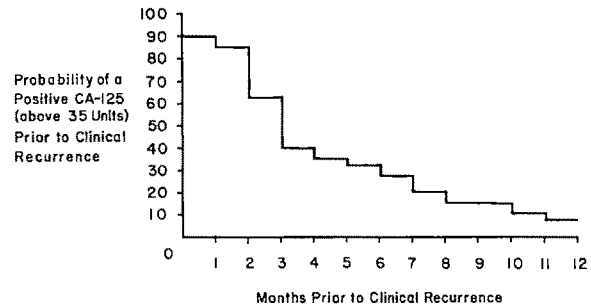


Fig. 3. CA 125 lead time for the 35 patients with clinical recurrences.

second-look procedures.^{6,7} Although a CA 125 level of <35 U/ml was associated with intraperitoneal tumor in about 50% of cases, the tumor was always of limited size. A CA 125 level of >35 U/ml consistently indicated intraperitoneal tumor.^{6,7} The present study was undertaken to determine whether the CA 125 assay could predict if and when a patient, after completion of primary therapy and second-look operation, would suffer a clinical recurrence.

The CA 125 assay obtained immediately before a second-look surgical procedure was highly prognostic for clinical recurrence, with a sensitivity of 94% and a specificity of at least 88%. With longer follow-up after this study was completed, the two other cases with elevated CA 125 levels have recurred, thus improving the specificity. Patients with a CA 125 level of ≥35 U/ml had a 60% chance of clinical recurrence within 4 months, while patients with levels of <35 U/ml had a 5% chance of recurrence over the same time interval. The CA 125 level had prognostic value for up to 1 year. Patients with an elevated assay at the second-look procedure are at highest risk for clinical recurrence in the first 4 months, while patients with CA 125 levels of <35 U/ml have increasing risk for recurrence after the first 4 months.

Serial CA 125 levels obtained periodically after second-look operations were strong predictors of clinical outcome. Distinct monitoring profiles were observed and were very different for patients remaining free of tumor and for those suffering clinical recurrence. Assay levels rose steadily or remained elevated for over 90% of the clinical recurrence group and fell steadily for over 80% of the group with no clinical evidence of disease.

The CA 125 assay became elevated before clinical recurrence in 94% of instances. Only 10% of patients remaining clinically free of tumor had elevated assays over comparable time intervals. (Both these patients subsequently recurred.) In one patient the single elevation was in the immediate postoperative period. Preliminary data have suggested that a surgical procedure may cause transient CA 125 elevations. It may therefore

be prudent to exercise caution in interpreting CA 125 levels obtained shortly after the operation.

The median lead time was 3 months. The CA 125 levels in this study were obtained at irregular intervals corresponding to individual investigators' follow-up protocols. It is likely that if CA 125 determinations were made at regular and more frequent intervals the lead time would be longer. Thirty-five units per milliliter is an appropriate reference value for predicting clinical recurrence and providing lead time.

At present the therapeutic decisions after the completion of primary therapy for epithelial ovarian cancer are based on tumor status determined at the second-look operation and subsequent laparotomies. Such procedures are associated with morbidity, and their accuracy may be limited by adhesions from prior surgery. The disease of a proportion of women free of tumor at second-look operations will recur,² and many patients with residual tumor present at these procedures will enjoy prolonged survival free of clinical recurrence measurable in terms of years.^{1, 4-6, 8} In the report by Podratz et al.¹⁵ the 5-year survival for patients with tumors not exceeding 1 cm was 39%, not significantly different from those with microscopic disease (43%). Tumor status at the operation may therefore not be the best prognosticator nor consequently the best indicator of which patients need additional therapy. Surgical procedures define tumor status at a single point in time and cannot be performed serially. CA 125 levels may be obtained at frequent intervals with no morbidity; thus patients may be followed easily. Elevated CA 125 levels indicate patients who are going to have recurrence of disease and provide lead time before recurrence measurable in months. CA 125 levels may therefore permit the institution of therapy earlier than would otherwise be possible in those individuals destined to have recurrence.

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The relationship between capillary and venous glucose concentration during pregnancy

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Previous attempts to construct capillary equivalents of venous glucose values for prognostic purposes have failed. We examined the relationship between capillary and venous glucose concentration during pregnancy in 258 women who had samples taken at four different time intervals in relation to two different standardized meals. Capillary glucose concentration was determined with the Chemstrips bG and an Accu-Chek reflectance colorimeter and venous plasma glucose concentration was measured by the hexose kinase technique on an AutoAnalyzer. The capillary:venous relationship was constant over time for a given meal but the magnitude of the difference was affected by time. The capillary:venous relationship differed significantly between the two standard meals. The findings indicate that meal size and sampling time must be controlled for when one is attempting to construct capillary equivalents for venous derived norms. The failure of previous studies to control for these variables may explain their inability to construct useful capillary equivalents for venous glucose values. Our findings also indicate that attainment of venous norms with capillary specimens represent, in reality, tighter control. (AM J OBSTET GYNECOL 1986;155:61-4.)

Key words: Papillary glucose, venous glucose, pregnancy

Capillary glucose self-measurement has been welcomed into the armamentarium of diabetes care. Nowhere is this more evident than in pregnancy. The ability to perform frequent measurements at home during pregnancy has reduced maternal and neonatal morbidity, neonatal mortality, and thus the cost of pregnancy for women with diabetes.¹ Multiple studies have confirmed both a high correlation between glucose concentration measured by a variety of reflectance colorimeters and that obtained by use of the standard hexose kinase techniques and between capillary and venous glucose concentrations despite the differing measuring techniques,²⁻⁴ leading some to equate capillary and venous values in practice. However, these same studies show a wide range of capillary values for a given venous glucose concentration, so that the prediction of equivalent concentrations from one specimen or the other is seemingly futile. This variability may result from the failure of all previous studies during pregnancy (and most in the nongravid patient) to adequately account for the possible effect of meal size, the time of sample collection, and individual patient variability. In 1972,

Lind et al.⁵ reported they were unable to demonstrate any constant relationship between capillary and venous blood glucose concentrations as had been implied by the recommendations of the World Health Organization Expert Committee on Diabetes Mellitus. They noted that at fasting, capillary:venous differences were too trivial to be worth correcting, especially during pregnancy. At higher glucose concentrations, the ranges were so variable that a practical, standard correction factor could not be identified. Unfortunately, their study design had a few important limitations. First, the number of patients investigated was insufficient to provide a broad database. Second, since the 36 fasting pregnant women examined were different from the 70 women from whom samples were obtained 45 minutes after a 50 gm glucose meal, it was impossible to control for individual patient variation. Third, only one carbohydrate meal size was used, so the effect of differing meal sizes could not be examined. The purpose of this study was to better define the capillary:venous glucose concentration relationship by measuring glucose concentration simultaneously in capillary and venous specimens at multiple sampling intervals in a large group of pregnant women after two different, standardized carbohydrate meals.

Material and methods

The study population consisted of women receiving prenatal care at the University of Iowa. A total of 714 paired capillary:venous samples were examined from

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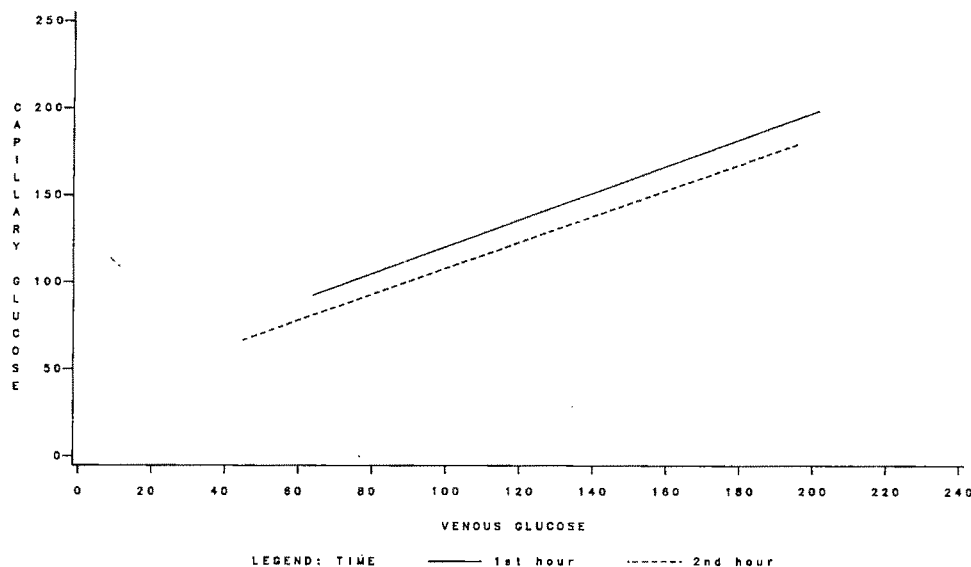


Fig. 1. Regression lines expressing the relationship between capillary and venous glucose concentrations during the glucose challenge tests after 50 gm meal. The slopes are not significantly different from each other.

Table IA. Mean capillary and venous glucose concentration 1 and 2 hours after ingestion of 50 gm of glucose (± 1 SD)

	Capillary (mg/100 ml)	Venous (mg/100 ml)
1 hr	133 $\pm$ 23	117 $\pm$ 24
2 hr	104 $\pm$ 22	95 $\pm$ 22

250 women. First 159 patients undergoing routine diabetes screening had paired capillary and venous specimens drawn at 1 and 2 hours after ingesting 50 gm of glucose (Trutol). Second, 99 women with a previous abnormal 1-hour screening test result (≥ 140 mg/100 ml) undergoing a 100 gm 3-hour oral glucose tolerance test had paired capillary and venous samples obtained at each of the four time intervals. Venous plasma glucose was measured on an AutoAnalyzer with the use of the hexose kinase technique. Capillary blood glucose concentration was measured by use of Chemstrips bG (glucose oxidase:peroxidase technique) and an Accu-Chek reflectance meter (each from Boehringer Mannheim Diagnostics, Inc.). All capillary glucose measurements were performed by three nurses specifically trained in the appropriate technique. Interassay variation among nurses during the training period was within 5%. An additional 127 venous samples from a different group of pregnant women being screened for diabetes were examined by both hexose kinase and glucose oxidase:peroxidase techniques to evaluate the accuracy of the Accu-Chek system in our hands when compared with the laboratory standard.

Regression analyses were applied to the pairs at each

time interval and the line of best fit predicting capillary concentration from venous concentration derived. An analysis of covariance was then performed to compare the slopes and intercepts within and among the times of the two tests blocking for the size of the meal, the sample time, and the patient.

Results

The correlation coefficient between hexose kinase and glucose oxidase:peroxidase techniques was high, 0.961. However, the resulting means (± 1 SEM), 112 ± 0.9 versus 108 ± 0.9 mg/100 ml, differed significantly ($p < 0.001$), with the Accu-Chek determination a mean of 3.7% below the standard hexose kinase technique. Thus technique alone resulted in a small underestimate of the capillary glucose concentration.

The mean capillary and venous glucose concentration (± 1 SD) for the 1- and 2-hour pairs after the 50 gm meal are listed in Table IA. The line of best fit for each time (Fig. 1) was:

$$\text{CAP 1} = (\text{V}) \times 0.771 + 43.126$$

$$\text{CAP 2} = (\text{V}) \times 0.746 + 33.404$$

The slopes of these two lines were not significantly different. This indicates that the relationship between capillary and venous glucose concentration was similar at the two time intervals. However, the intercepts were significantly different ($p < 0.001$). The impact of sampling time can best be illustrated by inspection of the respective intercepts after the equations are converted to equal slopes:

$$\text{CAP 1} = (\text{V}) \times 0.826 + 36.661$$

$$\text{CAP 2} = (\text{V}) \times 0.826 + 25.785$$

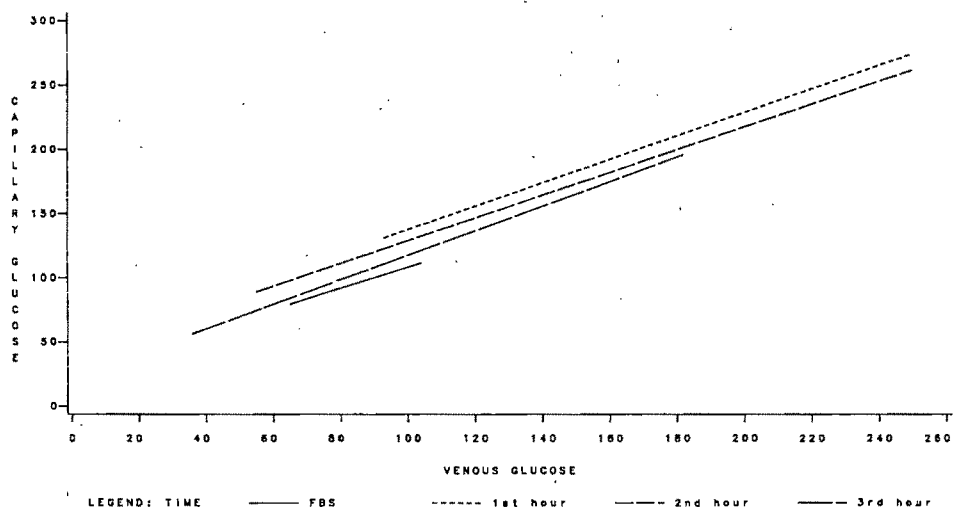


Fig. 2. Regression lines expressing the relationship between capillary and venous glucose concentrations during a 3-hour oral glucose tolerance test after a 100 gm meal. The slopes are not significantly different from each other. *FBS* = Fasting blood sugar.

The mean concentrations (± 1 SD) at each time interval for women receiving the 100 gm glucose meal are listed in Table IB. A total of 16.2% (16 of 99) were abnormal by standard criteria used at the University of Iowa.⁶ The regression lines of best fit were (Fig. 2):

$$\begin{aligned} \text{CAP F} &= \text{V} \times 0.784 + 28.515 \\ \text{CAP 1} &= \text{V} \times 0.899 + 47.648 \\ \text{CAP 2} &= \text{V} \times 0.867 + 41.922 \\ \text{CAP 3} &= \text{V} \times 0.942 + 22.88 \end{aligned}$$

As after the 50 gm meal, there were no significant differences among the slopes, but the intercepts were significantly different ($p < 0.001$). Thus the relationship between capillary and venous glucose concentration after the 100 gm meal may be best described as:

$$\begin{aligned} \text{CAP F} &= \text{V} \times 0.963 + 14.637 \\ \text{CAP 1} &= \text{V} \times 0.963 + 38.164 \\ \text{CAP 2} &= \text{V} \times 0.963 + 30.008 \\ \text{CAP 3} &= \text{V} \times 0.963 + 22.034 \end{aligned}$$

The CAP 1 and CAP 2 slopes for the 50 and 100 gm meals were significantly different ($p < 0.05$) from each other. Therefore, the size of the meal altered the relationship between capillary and venous glucose concentrations.

Comment

The high correlation between capillary and venous glucose concentration and the accuracy of various reflectance meters including the Accu-Chek have been well documented.²⁻⁴ Thus it was quite surprising that there had been no previous investigation of the relationship between the capillary and venous glucose concentrations with control for potential variables, such as meal size and the time after the meal when the speci-

Table IB. Mean capillary and venous glucose concentration during a 100 gm oral glucose tolerance test (± 1 SD)

	Capillary (mg/100 ml)	Venous (mg/100 ml)
Fasting	91.3 $\pm$ 12	80.0 $\pm$ 7
1 hr	189.0 $\pm$ 36	157.1 $\pm$ 33
2 hr	155.6 $\pm$ 33	131.1 $\pm$ 35
3 hr	116.9 $\pm$ 31	99.8 $\pm$ 31

men was obtained. Our study indicates that both of these variables are significant and must be accounted for in any attempt to derive the capillary equivalent of a venous value. It is not therefore surprising that previous attempts to construct such equivalent values for diagnostic purposes have failed.⁵ In addition, our findings underscore a popular misconception that the capillary specimen represents whole blood. Capillary glucose measurements as determined by the Chemstrips bG are not whole blood determinations but rather a red blood cell-free ultrafiltrate. The red blood cells do not reach the chemically active layer and are for the most part wiped off. Measurements of glucose concentration by this technique are affected by hematocrit, but not to a clinically significant degree (between 20% and 80%). The capillary glucose concentration exceeded the venous concentration by a mean of 15.6% over all examined time points despite an inherent technique bias. This red blood cell-free ultrafiltrate is more analogous to plasma/serum than whole blood. This observation supports technical reports from the manufacturer concerning the Chemstrip bG (unpublished data). Last, our findings have an important corollary. Because the capillary glucose concentration exceeds the

venous concentration even at relatively low fasting levels, clinical goals based on venous studies, if achieved with the use of capillary measurements, represent, in reality, a tighter degree of glucose control.

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Insulinoma complicating pregnancy: Case report and review of the literature

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A 24-year-old woman, gravida 1, para 0, experienced recurrent attacks of headaches and bizarre behavior from the sixth week of gestation onward. Three days before confinement, she lapsed into coma and was delivered of a normal child. Plasma glucose, insulin, and C-peptide levels were diagnostic of insulinoma. Subsequently, after she spontaneously regained consciousness, a pancreatic tumor was removed at laparotomy with complete resolution of symptoms. The problems of diagnosing insulinoma during pregnancy are discussed. (*AM J OBSTET GYNECOL* 1986;155:64-5.)

Key words: Insulinoma, pregnancy, hypoglycemia, glucose level in pregnancy.

Insulinoma is a rare pancreatic tumor, and its incidence during pregnancy is very uncommon, with only seven cases having been previously reported in the literature.^{1,2} We report herein the case of a young woman who suffered from hypoglycemic attacks during pregnancy due to an insulinoma with deterioration to coma at confinement.

Case report

A 24-year-old woman, gravida 1, para 0, was healthy until the sixth gestational week when she started to experience recurrent episodes of bizarre behavior and headache, with intermittent confusion and difficulty on waking up in the morning. The symptoms improved after breakfast. The following two trimesters were characterized by attacks of stupor, which were treated with carbamazepine (Tegretol), with no improvement.

Three days before confinement she was found by her husband in a state of unconsciousness and brought to a local clinic. On physical examination she was in a deep coma, and the plasma glucose level was found to be 35 mg/100 ml. Three days later a spontaneous vaginal delivery, while the patient was in a coma, resulted in the birth of a male infant weighing 3500 gm. The infant was healthy at birth and developed normally during the following months. Two days after confinement she was transferred to another hospital where a mild clinical improvement manifested by response to painful stimuli was noted and the range of plasma glucose level was 30 to 50 mg/100 ml, despite intravenous infusions of 50% glucose. Two days later she was admitted to our hospital for further workup.

The patient's past medical and obstetric history was unremarkable; on physical examination, other than the comatose state and mild bloody vaginal discharge, there were no relevant findings. Plasma glucose level, immunoreactive insulin level, plasma glucose/immunoreactive insulin ratio, and C-peptide level in five blood samples are detailed in Table I. The findings of inappropriate hyperinsulinism accompanied by detectable C-peptide levels were suggestive of insulinoma. Other hormone levels, liver and kidney function tests, and a

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Table I. Plasma glucose, immunoreactive insulin, C-peptide, and immunoreactive insulin/plasma glucose ratios of five random blood samples during first 2 days of hospital admission

	<i>Blood sample</i>				
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
Plasma glucose (mg/100 ml) (normal, 60-110)	10	48	86	55	77
Immunoreactive insulin (μ U/ml) (normal, <20)	145	40	104	20	57
C-peptide (pmol/ml) (normal, 0.18-0.63)	0.24	0.02	0.77	0.62	0.67
Immunoreactive insulin/plasma glucose ratio (noninsulinoma <0.3 < insulinoma)	14.5	0.83	1.21	0.36	0.74

complete blood count were within the normal range. Electroencephalography was compatible with severe metabolic encephalopathy; computerized tomographic examination of the brain showed no abnormalities, and abdominal ultrasonic examination revealed no abnormal findings in the pancreas. In view of the severe brain damage it was decided, at this point, not to pursue further investigation and the patient was treated with diazoxide and glucose. A gradual clinical improvement occurred during the following weeks, leading to a state of a mild affective disorder and right hemiparesis. Abdominal computerized tomography and celiac angiography did not reveal a pancreatic tumor. On laparotomy, a 3 cm pancreatic tumor was extirpated and histologically confirmed as insulinoma. After operation, all hypoglycemic symptoms subsided with no further treatment.

Comment

Metabolic requirements during normal pregnancy modify gradually concomitant with the increase of metabolically active steroids and fuel demands; this modification is fully expressed during the second half of gestation.³ These gestational interactions create a pattern of "accelerated starvation" when food is withheld, alternating with "facilitated anabolism" during the fasting state. Accordingly, plasma glucose levels fluctuate between near hypoglycemia in the fasting state and relative hypoglycemia after food ingestion. Our patient

exemplifies this pattern: Before pregnancy the insulinoma was asymptomatic and was "unmasked" only during periods of "accelerated starvation," during which the maternal mechanisms for maintenance of euglycemia are markedly diminished. A review of previously reported cases of insulinoma during pregnancy indicates no consistent pattern of clinical presentation; hypoglycemic symptoms may deteriorate, as in the case of our patient, or may actually improve. This variability is not unexpected because of the heterogeneous biologic behavior of insulin-secreting islet cells tumors. Clearly, the message in our case is that when severe hypoglycemia occurs during pregnancy, especially in conjunction with neurological symptoms, the possibility of insulinoma should be considered. This diagnosis can be easily confirmed by a simultaneous determination of plasma glucose, insulin, and C-peptide levels in the fasting state.

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Fetal acid-base balance in low-risk patients in labor

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The fetal acid-base balance from scalp blood in the early and late first stage of labor and from cord arterial blood was assessed in 120 low-risk patients only monitored electronically for 15 minutes at admission. The mean scalp blood pH values were 7.332 at a cervical dilatation of 5 cm and 7.335 at 10 cm cervical dilatation. A low pH value (<7.25) was found in 14 patients; five (4.2%) of them had fetal distress. Stethoscopic auscultation failed to identify these cases. Another nine patients had transient abnormal acid-base balance (a check value within 30 minutes was normal), and the fetal heart rate trace (obtained immediately after the sampling) showed no ominous changes in these cases. It is concluded that an abnormal fetal acid-base balance is not uncommon in a low-risk population in labor. However, a critical evaluation of the acid-base balance is necessary, since in most cases the abnormal acid-base balance is transient and of a respiratory, innocuous type without concomitant fetal distress. (*AM J OBSTET GYNECOL* 1986;155:66-9.)

Key words: Fetal acid-base balance, low-risk labor

Fetal acid-base balance in blood obtained from the scalp in labor or from the cord at delivery is an important parameter for assessing fetal well-being. However, defining normal ranges for fetal acid-base values might be problematic, and considerable discrepancies exist between different reports.¹ Apart from methodologic problems, the "normal" population from which the samples have been taken is defined in different ways. Most workers have excluded prepartum and intrapartum complications from the study population, have retrospectively studied cases with normal Apgar scores at delivery, or have excluded cases with a low pH.²⁻⁵ In other materials the criterion for normality has been a normal electronic fetal heart trace throughout labor.⁶ Thus the incidence of an abnormal fetal acid-base balance in an unselected population cannot be defined from the above-mentioned studies.

In clinical practice the management of patients in labor will often be guided according to a risk classification during the antenatal period. Risk assessment profiles have been recommended by some authors for selecting patients for monitoring.⁷ It is believed that after such a selection a low-risk group is created with a very low risk for fetal acidosis in labor. Unfortunately, few studies have been designed to assess this risk, and the validity of risk profiles for predicting fetal problems

in labor have been challenged.^{8,9} This dilemma has led many departments to monitor all patients regardless of risk.¹⁰

In the present study we monitored the fetal well-being during labor and at delivery in a low-risk population by repeated fetal scalp blood and cord arterial blood acid-base balance determinations. These parameters were used to evaluate the risk for fetal acidosis in low-risk patients. Electronic fetal monitoring was normally not used so as not to influence the management but was instituted in cases with low pH values. The incidence of fetal distress was calculated from the number of cases with low pH values, ominous fetal heart rate changes, and fetal depression at birth.

Patients and methods

One hundred twenty low-risk patients (54 nulliparas, 66 multiparas) with cephalic presentation at term (37 to 42 weeks) participated in the study at the University Department, Kandang Kerbau Hospital, National University of Singapore. This study was performed in the low-risk labor ward where patients with an uncomplicated pregnancy and no evidence of risk factors at admission (such as vaginal bleeding or malpresentation) were managed.

On admission to the labor ward the patients were electronically monitored for 20 minutes (admission test) as a part of another study. The numerical display, and the recording were concealed. The volume was turned off, and only the lamp indicating an optimal recording was visible. The trace was kept in an envelope, and the outcome of the test was not revealed during labor or delivery so as not to influence the management.

The fetal well-being was assessed by measuring the

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Table I. Acid-base balance in scalp blood at a cervical dilatation of 5 and 10 cm and in cord arterial blood at birth (mean values $\pm$ SD)

	Cervical dilatation				Cord arterial blood	
	5 cm		10 cm		Mean	SD
	Mean	SD	Mean	SD		
pH	7.332	0.05	7.335	0.07	7.292	0.07
PCO ₂ (mm/Hg)	46.85	8.15	46.57	7.36	50.56	9.47
PO ₂ (mm/Hg)	25.00	12.69	24.06	13.58	21.05	8.71
HCO ₃ (mmol/L)	24.15	2.67	23.93	2.88	23.08	2.69

Table II. Distribution of pH values in scalp blood at a cervical dilatation of 5 and 10 cm and in cord arterial blood at birth

pH	Cervical dilatation				Cord arterial blood	
	5 cm		10 cm		n	%
	n	%	n	%		
<7.100	0	—	0	—	2	1.7
7.100-7.149	1	0.8	2	1.7	2	1.7
7.150-7.199	1	0.8	1	0.9	5	4.2
7.200-7.249	5	4.2	4	3.4	22	18.3
7.250-7.299	24	20.0	21	17.8	30	25.0
7.300-7.349	42	35.0	48	40.6	35	29.1
7.350-7.399	41	34.2	32	27.1	18	15.0
7.400-7.449	6	5.0	8	6.8	6	5.0
>7.450	0	—	2	1.7	0	—
Total	120	100.0	118*	100.0	120	100.0

*Two patients had a cesarean section for fetal distress before reaching a cervical dilatation of 10 cm.

acid-base balance in scalp blood obtained at a cervical dilatation of 5 and 10 cm. The acid-base balance was also measured at birth in cord arterial blood obtained by puncture of the vessel before the first breath. In 60 patients a maternal capillary blood sample from the finger tip was collected at the time of fetal blood sampling, but in the rest of the patients only when the fetal pH was <7.25. All samples were taken by one of us. The samples were analyzed in an AVL automatic gas check.

To minimize sampling error, (1) all samples were obtained by one of us, (2) the samplings were performed with the patients in the left lateral position, (3) the blood was collected directly into the glass capillary tube for analysis, (4) the sample was rejected unless the glass capillary tube was filled easily, (5) no patients had a pronounced caput, (6) the samples were analyzed within a few minutes, (7) conduction anesthesia for pain relief (such as epidural block) was not given, and (8) the cord artery was punctured immediately after birth and the blood collected before the first breath.

The fetal heart rate was auscultated for half a minute by stethoscope every 30 minutes until delivery. For ethical reasons continuous electronic fetal heart rate monitoring was instituted if a fetal pH from the scalp was

<7.25, if an abnormal fetal heart rate was auscultated, or if thick meconium was detected. A preacidotic fetal pH (7.20 – 7.24) was checked within 30 minutes with a new sample if the fetal heart rate trace was reassuring.

We considered fetal distress to be present if the fetal scalp pH was low (<7.25), if the fetal heart rate trace was ominous at the time of the low pH, and if the newborn infant had either an Apgar score <7 at 1 minute and/or a cord artery pH <7.15.

Results

Table I shows the mean values for pH, PCO₂, PO₂ and bicarbonate in early and late first stage of labor and at birth. There was no difference in pH values in the scalp blood between nulliparas and multiparas. The length of labor from 5 to 10 cm cervical dilatation had no influence on the acid-base balance. In the nulliparous group the cord pH was lower (7.276) than in the multiparous group (7.304).

The distribution of pH values is shown in Table II. At a cervical dilatation of 5 cm, seven patients (5.8%) had a pH value <7.25. Two of these cases were delivered by cesarean section for fetal distress: one case had a scalp blood pH of 7.113 (PCO₂ 52.4, HCO₃ 16.2), and the other had a pH value of 7.241 (PCO₂ 55.9, HCO₃

Table III. Fetal acid-base balance in cases with transient low values (values obtained should be checked within 30 minutes)

Patient No.	pH	P_{CO_2} (mm/Hg)	H_{CO_3} (mmol/L)	Check of pH value	Cord arterial pH	Remarks
1*	7.180	67.7	23.3	7.276	7.249	Unknown reason
2*	7.212	59.3	23.1	7.293	7.230	Unknown reason
3*	7.228	68.2	27.6	7.299	7.283	FHR bradycardia before sampling
4*	7.242	57.3	23.9	7.272	7.304	Thick meconium
5*	7.249	66.4	28.3	7.313	7.197	FHR bradycardia before sampling
6†	7.208	47.0	18.1	NA	7.232	Crowning of the fetal head
7†	7.226	59.5	23.9	7.287	7.313	Unknown reason
8†	7.234	56.2	23.0	NA	7.405	Unknown reason
9†	7.244	53.2	22.3	NA	7.240	Maternal acidosis (pH 7.313)

NA = Not assessed, FHR = fetal heart rate.

*Sampling at 5 cm cervical dilatation.

†Samplings at 10 cm cervical dilatation.

23.7) and a fetal heart rate trace immediately afterwards showing bradycardia, reduced baseline variability, and pronounced late decelerations which indicated performance of a cesarean section without further delay. At a cervical dilatation of 10 cm, seven patients (5.8%) had pH values <7.25, and three of these cases had fetal distress: pH 7.115 (P_{CO_2} 55.2, H_{CO_3} 17.7), pH 7.154 (P_{CO_2} 67.5, H_{CO_3} 23.0), and pH 7.194 (P_{CO_2} 77.3, H_{CO_3} 17.7), respectively. One had a spontaneous delivery whereas two had forceps delivery.

The remaining five cases in the 5 cm group and four in the 10 cm group had a normal fetal heart rate trace (obtained immediately after scalp blood sampling according to the protocol) and a normal check pH value within 30 minutes either from the scalp or from the cord if delivery occurred during that period (Table III). There were no signs of fetal distress among the nine patients with transient low scalp blood pH values. The acidosis was of a respiratory type in all cases but one (case 6). In four cases the possible cause was fetal bradycardia (observed on the admission test) after a vaginal examination (2), crowning of the fetal head (1), and maternal acidosis (1). No obvious cause was apparent in the remaining five cases. All patients but one (with cesarean section for malposition) had a spontaneous vaginal delivery, and all newborn infants had normal cord arterial pH values and normal Apgar scores at birth.

Stethoscopic auscultation findings were normal in the five cases with fetal distress as well as in the group with transient, low scalp pH values. On the other hand, stethoscopic auscultation suggested fetal distress in three other cases, but fetal heart rate traces and scalp blood pH values were normal.

Comment

Since the pioneer work of fetal blood sampling in labor,¹¹ a considerable number of studies on fetal acid-base balance in "normal" labor are published. However,

it is notable that great discrepancies exist between reports of mean values for the different parameters. The mean fetal scalp blood pH in normal labor was within a range from 7.30 to 7.40 in the early first stage of labor and from 7.29-7.37 in the last first stage of labor in the surveyed literature.¹² Lumley et al.¹ found that three different groups of results could be distinguished statistically in reports of normal pH values: pH 7.29-7.30, 7.32, 7.35-7.38. Consequently the normal range also differed considerably.

In this material the mean pH values were similar in early and late first stage of labor (7.332 and 7.335, respectively) and were not influenced by parity. The acid-base balance values in cord arterial blood were similar to that of a recent report on patients with an uncomplicated term vaginal delivery.¹³ The distribution of pH values (Table III) shows that scalp blood pH values <7.30 are not uncommon in low-risk patients; such a value was found in 59 (24.8%) of a total of 238 samples in the first stage of labor. It is generally agreed that a scalp blood pH value <7.25 is abnormal and considered as preacidotic if it is between 7.20-7.24 and acidotic <7.20.⁵ The incidence of low pH values in scalp blood in this study was 5.8% and 6.0% in early and late first stage of labor, respectively.

In this study, five cases fulfilled the criteria for fetal distress with a low scalp pH, an ominous fetal heart rate trace, and a low Apgar score (at 1 minute) and/or a low cord arterial pH (<7.15) at birth.

Another nine cases had a low scalp blood pH but had no ominous fetal heart rate changes when monitored immediately after the sampling. A check sample within 30 minutes showed a normal acid-base balance either from the scalp or from the cord artery (in three cases delivered within the time period). These cases with transient low pH values (Table III) constitute an interesting group demonstrating that pH values <7.25 can be obtained in a considerable number of patients without any signs of concomitant fetal distress. These find-

ings stress the necessity to critically evaluate acid-base values and not rush into intervention on a single sample. It adds strength to electronic fetal monitoring, because if the fetal heart rate trace shows no ominous changes, the risk for fetal distress appears to be small. In two of the cases the possible cause for low pH value was a prolonged fetal heart rate bradycardia (observed on the admission test) following a vaginal examination. In another case the pH sampling was done at crowning of the fetal head. It is well known that the fetal scalp blood pH decreases in the late first stage of labor, and this decrease is predominantly of metabolic origin.² In another case maternal acidosis may have been responsible for the low fetal pH. Rooth¹⁴ found a close relationship between maternal and fetal acid-base balance in labor. However, in the remaining five cases no obvious cause for the low pH could be found.

In this study 76% of the patients had early amniotomy adhering to the normal practice in our labor ward. This allows for early detection of meconium staining in the liquor,¹⁵ shortens labor,^{16, 17} and permits direct fetal heart rate or intrauterine recording to be undertaken.¹⁸ Early reports suggested the possibility of altered fetal heart rate pattern and reduction in pH with such a practice,¹⁹ but this was not substantiated by subsequent reports by other authors.¹⁷ Our experience supports the latter view.

In this population the fetal heart rate was intermittently auscultated for 30 seconds every 30 minutes in the first stage and every 5 minutes in the second stage of labor and was electronically monitored only on indication: low scalp blood pH (<7.25), abnormal fetal heart rate on auscultation, or appearance of thick meconium. An attempt was made to assess the accuracy of stethoscopic auscultation in a low-risk population in which the fetal well-being was well assessed by acid-base determinations. None of the five cases with fetal distress was detected by auscultation. Three other cases with abnormal fetal heart rate on auscultation had normal fetal heart rate traces, normal scalp blood pH values, and no signs of fetal distress. These results indicate that stethoscopic auscultation as practiced in some busy labor wards such as ours is not as adequate as electronic fetal monitoring in detecting fetal distress. Auscultation of the fetal heart rate up to 1 minute every 15 minutes immediately after a contraction in the first stage and after every contraction during the active second stage of labor would be preferable. However, it is unlikely that this had changed the outcome of these results in a decisive way, since mild to moderate decelerations as well as the baseline variability are difficult to determine by auscultation.

In conclusion, the results in this study suggest that

abnormal fetal acid-base balance (with a scalp blood pH <7.25) in the first stage of labor can be found in a sizable proportion of low-risk patients. Five (4.2%) cases had fetal distress with a low pH value, concomitant ominous fetal heart rate changes, and fetal depression at birth. However, in most of the cases the abnormal acid-base balance was of a respiratory type (Table III), being transient and innocuous. A critical evaluation of low fetal pH values seems advisable to detect such cases, since other causes for the disturbance than fetal distress should be looked for.

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The role of platelet-activating factor in human fetal lung maturation

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The presence of the potent bioactive glycerophospholipid 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) in a lamellar body-enriched fraction of amniotic fluid from women in labor has prompted the present investigation to examine the fetal lung as the possible tissue source of this platelet-activating factor. The metabolism of platelet-activating factor was assessed in an organ culture system in which human fetal lung tissue (12 to 16 weeks) was incubated for 6 to 7 days. During this period, the type II pneumocytes differentiate and surfactant glycerophospholipid biosynthesis is greatly enhanced. The initial specific activity of the platelet-activating factor biosynthetic enzyme lyso-platelet-activating factor:acetyl-coenzyme A acetyltransferase is two to three times greater in microsomes prepared from fetal lung than in those from the fetal liver or kidney. The specific activity of acetyltransferase in lung tissue increased twofold after 6 days in organ culture. A similar increase in acetyltransferase activity was found in the lamellar body-enriched ($18,000 \times g$) subcellular fraction of fetal lung. The activity of the major platelet-activating factor-inactivating enzyme platelet-activating factor acetylhydrolase did not change significantly in the lung explants during the incubation period. Associated with the enhanced platelet-activating factor biosynthetic activity in the fetal lung was an increase in the platelet-activating factor concentration, from 17 to 37 fmol/mg of protein between days 0 and 6 of culture. Corresponding increases of 3.3- and 3.8-fold in the concentrations of the platelet-activating factor lipid precursors lyso-platelet-activating factor and the 2-acyl congener were found after 6 days in culture. A reciprocal relationship was found between platelet-activating factor and glycogen content as the lung tissue matured in vitro; specifically, as the platelet-activating factor level increased, glycogen decreased from 340 to 77 μg /mg of protein. We suggest that platelet-activating factor may mediate the onset of glycogenolysis in fetal lung tissue similar to that in the isolated, perfused rat liver (Shukla SD, Buxton DB, Olson MS, Hanahan DJ. Acetylglucyl ether phosphorylcholine. A potent activator of hepatic phosphoinositide metabolism and glycogenolysis. *J Biol Chem* 1983; 258:10212-4). The increased rate of glycogen degradation may serve as the carbon and energy source for the increased synthesis of glycerophospholipids required for surfactant production. The platelet-activating factor may also be secreted from the type II pneumocytes, in association with the surfactant, into the amniotic fluid where it can stimulate prostaglandin E_2 formation in amnion tissue. (*AM J OBSTET GYNECOL* 1986;155:70-5.)

Key words: Platelet-activating factor, lung maturation, phospholipids, human lung organ culture, glycogen metabolism

Previously, we reported that platelet-activating factor (PAF, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), was present in amniotic fluid from women in labor but absent in samples obtained from women at term pregnancy but not in labor.¹ Nishihara et al.² have recently confirmed and extended these observations.

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The PAF was localized to a significant degree in a $35,000 \times g$ pellet of amniotic fluid.¹ This fraction is also rich in lamellar bodies. On the basis of these findings we suggest that the fetal lung may be a significant source of the PAF present in amniotic fluid. Although the enzyme involved in PAF biosynthesis, lyso-PAF:acetylcoenzyme A acetyltransferase, has been shown to be present in the adult rat lung,³ little information is available on the activity of this enzyme in fetal tissues.

Of the numerous biologic functions attributed to PAF,³ its activation of glycogenolysis is most pertinent to the present investigation.⁴ Addition of nanomolar amounts of PAF to the perfusate of the isolated adult rat liver rapidly induced the release of glucose (via breakdown of glycogen). Glycogenolysis has also been postulated to be of great significance in the develop-

ment of fetal lung. Both morphologic and biochemical investigations⁵⁻⁷ have demonstrated the presence of abundant glycogen particles in the cytoplasm of undifferentiated ductal lung epithelial cells of various species throughout most of gestation. At the time of type II pneumocyte development, the appearance of lamellar bodies is accompanied by the disappearance of glycogen. Concurrent with the breakdown of pulmonary glycogen during gestation is an increase in the rat lung in the content and synthesis of disaturated phosphatidylcholine, the major glycerophospholipid of surfactant. This characteristic relationship between glycogen disappearance and phosphatidylcholine biosynthesis has been demonstrated in fetal lung tissue⁶ and it has been suggested that glycogen degradation may contribute both the carbon units and the metabolic energy necessary for surfactant glycerophospholipid biosynthesis.^{6,7}

In the present communication, we report an increase in the concentration of PAF during a 6-day culture period of human fetal lung explants. The acetyltransferase as well as the acetylhydrolase, the enzyme that inactivates PAF, in the human fetal lung was assayed after various time periods in culture. The presence of the various lipid substrates that are utilized for PAF biosynthesis was determined in human fetal lung tissue. The diminished glycogen content of the fetal lung was assayed during the culture period and related to the PAF content in this tissue.

Material and methods

Chemicals. 1-Alkyl-2-[³H]acetyl-glycero-3-phosphocholine (50 μ Ci/ μ mol) and 1-[³H]alkyl-2-lyso-glycero-3-phosphocholine (50 μ Ci/ μ mol) were provided by Dr. Fred Snyder, Oak Ridge, Tennessee. 1-[³H]Alkyl-PAF (115 Ci/mmol) and [³H]acetylcoenzyme A (500 mCi/mmol) were purchased from Amersham Corporation, Arlington Heights, Illinois. Fatty acid-poor, fraction V bovine serum albumin (No. A-6003), creatine phosphate, creatine phosphokinase, indomethacin, amyloglucosidase (No. A-7255), glucose diagnostic kit No. 510 and semisynthetic PAF were obtained from Sigma Chemical Co., St. Louis, Missouri. Unlabeled lyso-PAF was obtained from Calbiochem, La Jolla, California.

Culture techniques. Lung tissues were obtained aseptically from human abortuses of 12 to 16 weeks' gestational age in accordance with the Donors Anatomical Gift Act of the State of Texas. Consent in writing was obtained from the women undergoing abortion: the consent form and protocol were approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas, Texas.

The lung tissue was rinsed several times with sterile culture medium, Waymouth's MB752/1 containing 100 units/ml of penicillin, 100 μ g/ml of streptomycin, and

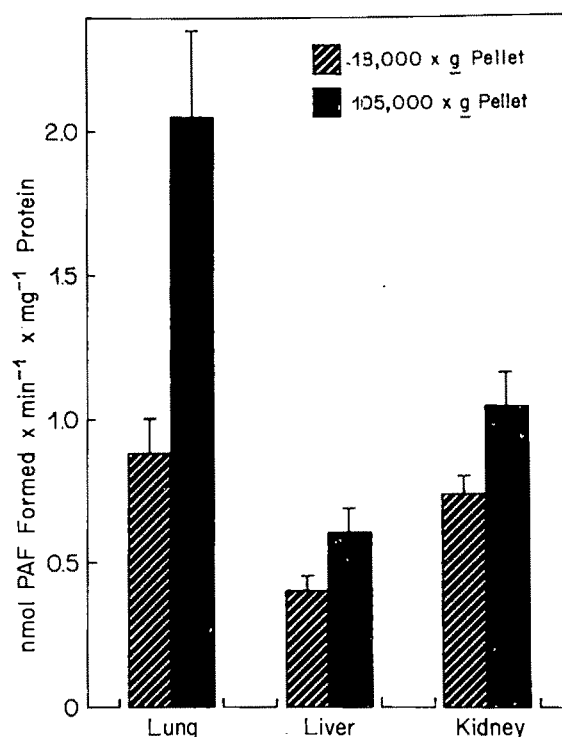


Fig. 1. Activity of acetyltransferase in various human fetal tissues. Values are the mean $\pm$ SEM from three separate experiments where lung, liver, and kidney were obtained from the same fetus. The enzyme was assayed as described in Material and methods.

0.25 μ g/ml of fungizone (all from Gibco, Grand Island, New York). Major airways and blood vessels were dissected from the lung and the remaining lung tissue was minced into small pieces (approximately 1 mm³) with a sterile razor blade.

Tissue pieces were placed on a square of sterile lens paper supported by a stainless steel grid in a plastic Petri dish 35 mm in diameter (Falcon, Oxnard, California). This dish contained 1 ml of tissue culture medium that was changed daily. Tissues were maintained at 37° C in an atmosphere of air (95%) and carbon dioxide (5%). Development of the human fetal lung tissue in culture has been described previously.⁸

Subcellular fractionation. The human lung explants were harvested after a designated incubation period and homogenized in five volumes of sucrose, 0.25 mol/L with the use of a Potter-Elvehjem vessel fitted with a teflon pestle. The suspension was centrifuged at 600 $\times$ g for 10 minutes to remove unbroken cells and cell debris. The supernatant fraction was centrifuged at 18,000 $\times$ g for 15 minutes to obtain a pellet containing mitochondria and lamellar bodies.⁹ The remaining supernatant fraction was centrifuged at 105,000 $\times$ g for 60 minutes to obtain the microsomal (pellet) and cytosolic fractions (supernatant), respectively.

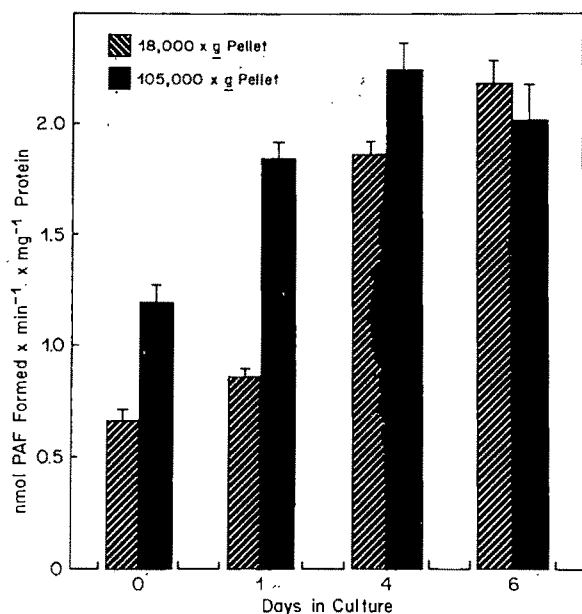


Fig. 2. Activity of acetyltransferase in cultured human fetal lung explants. Values are the mean $\pm$ SEM.

Enzyme assays. Activity of lyso-PAF:acetylcoenzyme A acetyltransferase was assayed by a modification of the procedure described previously.¹⁰ The reaction mixture contained Tris hydrochloride (30 mmol/L, pH 7.4 at 37° C), dithiothreitol (1 mmol/L), calcium chloride (10 μ mol/L), lyso-PAF (40 μ mol/L) suspended by sonication to give a final concentration of 0.025% bovine serum albumin, [³H]acetylcoenzyme A (250 μ mol/L, 6.4 μ Ci/ μ mol), and 40 to 60 μ g of protein. The incubations (0.25 ml total volume) were carried out for 15 minutes at 37° C and terminated by addition of methanol:chloroform:acetic acid (49:25:1). The reaction product, PAF, was identified by thin-layer chromatography on silica gel H plates. Reaction mixtures containing no added lyso-PAF served as blanks. The activity of acetyltransferase was linear with time to 20 minutes and with microsomal protein to 120 μ g.

The activity of PAF acetylhydrolase in cytosolic fractions was determined according to the method previously described.¹⁰ The reaction mixtures and blanks (containing boiled cytosol, 10 minutes at 100° C) were incubated for 20 minutes at 37° C and terminated by addition of 2 ml of chloroform:methanol (1:2). The assay was linear with cytosolic protein to 400 μ g and to 30 minutes.

Glycogen content of fetal tissues were determined by the procedure of Chan and Exton.¹¹ Glucose was determined by the glucose oxidase-peroxidase-O-dianisidine procedure (Sigma diagnostic kit No. 510). Protein was quantitated by the method of Lowry et al.¹²

Lipid extraction. Tissue (100 to 300 mg), designated

for quantitation of PAF and related lipids, was first homogenized in 1.5 ml of water (4° C, in polypropylene tubes) with a Polytron homogenizer. Aliquots were taken for protein determination and lipid extraction; lipids were extracted as described previously.^{1, 10} 1-[³H]Alkyl-PAF (3.5 nCi/31 fmol) was added to the extract to calculate recovery of lipid. The recovery ranged between 70% and 85% and the PAF concentration was corrected to 100%. PAF, lyso-PAF, and 1-alkyl-2-acyl-glycero-3-phosphocholine were separated by thin-layer chromatography as described.^{1, 10} The isolated PAF was further identified by the procedures previously reported.¹ The lyso-PAF and 1-alkyl-2-acyl-glycero-3-phosphocholine samples were chemically converted to PAF and quantitated as described previously.¹

Bioassay of PAF. A suspension of washed rabbit platelets was prepared as previously described¹ with the exception that the platelets were washed once in indomethacin (10 μ mol/L). PAF quantitation was conducted by the procedure previously described¹ with a dual-channel platelet aggregometer (Sienco Inc., Morrison, Colorado) at 37° C with constant stirring. A standard curve of aggregation versus authentic PAF concentration (10 to 200 pmol/L) was prepared for each assay group.

Results

Acetyltransferase and acetylhydrolase activities in fetal lung. In Fig. 1 the specific activities of acetyltransferase in matched sets of fresh human fetal lung, liver, and kidney tissue samples (day 0) are illustrated. The activity of acetyltransferase was predominantly in the microsomal fraction (105,000 $\times$ g pellet) of fetal lung; however, significant activity was present in the mitochondrial-lamellar body fraction (18,000 $\times$ g pellet, see Fig. 2). The specific activity of the enzyme in the microsomal fraction was 200 times greater than that found in the cytosolic fraction (105,000 $\times$ g supernatant, data not shown). The microsomal acetyltransferase activity in fetal lung was 3.3-fold and twofold greater than that present in fetal liver and kidney, respectively (Fig. 1).

Acetyltransferase in fetal lung was further characterized by determination of its kinetic properties. The optimum pH of the enzyme was found to be 7.5 at 37° C in 30 mmol/L Tris hydrochloride (data not shown). The apparent Michaelis-Menten constant (K_m) values were approximately 18.7 μ mol/L for lyso-PAF and 96 μ mol/L for acetylcoenzyme A with either [³H]acetylcoenzyme A or 1-[³H]alkyl-2-lyso-glycero-3-phosphocholine used as the radioactive tracer. The apparent maximum velocity was approximately 7.7 nmol $\times$ min⁻¹ $\times$ mg⁻¹ of protein for the microsomal enzyme from fresh (day 0) human fetal lung tissue.

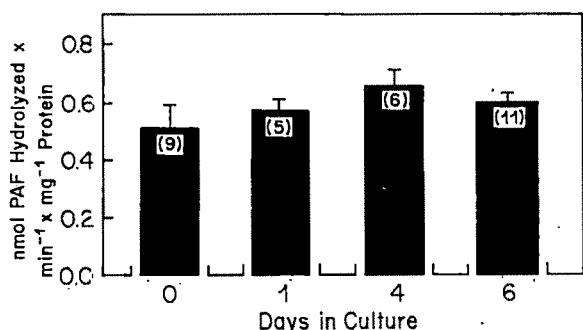


Fig. 3. PAF-acetylhydrolase activity in cultured human fetal lung explants. Acetylhydrolase was assayed as described in Material and methods. Values in parentheses are the number of determinations used to obtain specific activity ($\pm$ SEM) at each time interval.

On culture of human fetal lung explants, the type II pneumonocytes mature morphologically (that is, appearance of lamellar bodies and microvilli, decrease in glycogen granules) and biochemically (increased production of surfactant phospholipids).⁸ As is illustrated in Fig. 2, there is an increase in the activity of acetyltransferase in both the $18,000 \times g$ and $105,000 \times g$ pellet fractions during the 6-day culture period. The microsomal activity of the enzyme increased by 55% in the first 24 hours of culture and by 90% after 4 days. It is also apparent that a shift occurs from day 0, where the majority of PAF is synthesized in the endoplasmic reticulum ($105,000 \times g$ pellet), to day 6 in culture, where the specific activity of acetyltransferase found in the mitochondrial-lamellar body fraction ($18,000 \times g$ pellet) is similar to that microsomes (Fig. 2). Despite a variability of the specific activities of acetyltransferase between individual tissue samples, a consistent twofold to threefold increase in activity was found in fetal lung preparations after 4 to 6 days in culture.

It has been suggested that the change in the major PAF degradative enzyme acetylhydrolase in various tissues may regulate the concentration of PAF in tissues.³ We have assayed the activity of acetylhydrolase in the subcellular fractions of fetal lung tissue and found this enzyme to be localized primarily in the cytosolic fraction ($105,000 \times g$ supernatant, data not shown). The specific activity of acetylhydrolase remained constant throughout the 6-day incubation period (Fig. 3). Thus it would appear that acetylhydrolase would not contribute significantly to the increased PAF levels in the human fetal lung.

Concentrations of PAF and its lipid precursors. The PAF concentration in lung tissue increased by 95% after 24 hours in culture and remained at this level throughout the 6-day incubation (Fig. 4). This increase in PAF concentration during culture was also apparent when

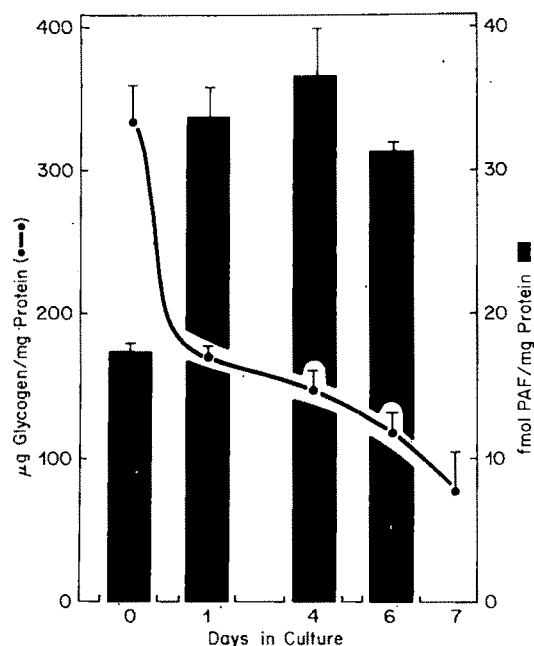


Fig. 4. Changes in the concentrations of PAF and glycogen in cultured human fetal lung explants. PAF values are the mean $\pm$ SEM of three experiments. Glycogen values are the mean of at least three separate determinations. PAF bioassay and glycogen analysis were performed as described in Material and methods.

expressed as the phospholipid molar ratio; thus PAF increased from 94 (day 0) to 221 (day 6) nmol/mol of phospholipid.

To support synthesis of PAF in the fetal lung, it is necessary that the lipid precursors be present in sufficient concentrations. Thus, in human fetal lung organ cultures, we determined the concentrations of lyso-PAF, the immediate precursor to PAF, and 1-alkyl-2-acyl-glycero-3-phosphocholine, a physiologic source of lyso-PAF through the action of phospholipase A₂. The tissue levels of both lyso-PAF and 1-alkyl-2-acyl-glycero-3-phosphocholine in the lung explants increased by 3.3-fold and 3.8-fold, respectively, during the 6-day culture interval (Table I). Although the concentrations of lyso-PAF and 1-alkyl-2-acyl-glycero-3-phosphocholine in the fetal lung explants represent a very small percentage of the total phospholipid, these levels are several orders of magnitude greater than that of PAF (Fig. 4). Furthermore, the lyso-PAF concentration in fetal lung can be calculated to be approximately 130 nmol/L. This value is considerably below the apparent K_m of the acetyltransferase for lyso-PAF ($18.7 \mu\text{mol/L}$, see above). Thus, because of the availability of lyso-PAF, there exists a possible regulation of acetyltransferase activity.

Glycogen content of fetal lung explants. The concentration of glycogen rapidly diminished by 46% within the first 24-hour period of culture (Fig. 4). Gly-

Table I. Concentration of PAF lipid precursors in human fetal lung explants

Lipid	Days in culture			
	0	1	4	6
Lyso-PAF (pmol/mg protein) (n = 5)	0.76 ± 0.13	0.81 ± 0.13	1.16 ± 0.26	2.50 ± 0.20
AAGPC (pmol/mg protein) (n = 6)	88 ± 9	108 ± 10	167 ± 27	336 ± 17

Values are mean of the specified number of determinations. Lyso-PAF and AAGPC (1-alkyl-2-acyl-glycero-3-phosphocholine) were chemically converted to PAF and quantitated by platelet aggregation bioassay as described in Material and methods.

cogenolysis continued throughout the remainder of the culture period although the rate decreased from an initial 155 μg of glycogen hydrolyzed $\times \text{mg}^{-1}$ of protein $\times 24 \text{ hr}^{-1}$ to 15 $\mu\text{g} \times \text{mg}^{-1}$ of protein $\times 24 \text{ hr}^{-1}$ after the first day. During the 6-day incubation period, a total of 1210 nmol of glycogen-glucose per milligram of protein is degraded by the fetal lung. From previous experiments with human fetal lung explants,^{8,13} it was computed that a total of 70 nmol of dipalmitoyl-phosphatidylcholine $\times \text{mg}^{-1}$ of protein $\times 6 \text{ days}^{-1}$ was synthesized. Thus the rate of glycogenolysis would appear sufficient to supply ample carbon units for both the glycerol backbone and fatty acid moieties for surfactant glycerophospholipid biosynthesis.

Comment

The source of the PAF in amniotic fluid from women in labor is uncertain but likely tissue sources include the fetal lung, the fetal kidney, and the amniotic membrane. We have previously detected PAF in the urine of newborn humans¹; thus the kidney may release PAF into the amniotic fluid via fetal urine. The human fetal kidney has considerable acetyltransferase activity (Fig. 1) and is therefore capable of producing PAF. The activity of a second PAF biosynthetic enzyme, 1-alkyl-2-acetyl-glycerol:cytidine-5'-diphosphate cholinephosphotransferase, has also been found in the adult rat kidney.³ The activity of this enzyme in the fetal lung is presently unknown. PAF synthesis occurs in the human amnion and is stimulated *in vitro* by the calcium ionophore A23187. The release of PAF from amnion into the media could not be demonstrated in these experiments.¹⁴ Thus amnion tissue is not a likely source of PAF in amniotic fluid. Localization of a significant proportion of PAF in the lamellar body fraction (35,000 $\times \text{g}$ pellet) of amniotic fluid¹ may be suggestive that the lung is the primary source of amniotic fluid PAF since lamellar bodies are a secretory product of lung type II pneumonocytes. These findings are consistent with the recent reports of PAF-like activity in rat¹⁵ and dog¹⁶ pulmonary lavage fluid.

Type II pneumonocytes differentiate in the human lung only after the twenty-fourth week of gestation;⁵ however, in the human lung organ culture system, the epithelial lining rapidly differentiates to resemble the

type II cell morphologic features.⁸ Several biochemical and morphologic events characterizing this transition include the disappearance of glycogen and the synthesis and accumulation of surfactant phospholipids in fetal lung explants in culture.^{3,6} Synthesis of disaturated phosphatidylcholine was increased by 80% in human fetal lung explants after 6 days in culture as measured by [³H]choline incorporation.⁸ Bourbon et al.⁶ radiolabeled the glycogen stores of 18.5-day rat fetuses with [¹⁴C]glucose, then analyzed the rates of glycogen disappearance and glycerophospholipid formation in cultured lung tissue. A significant proportion of radiolabel was recovered in disaturated phosphatidylcholine and in the second most abundant phospholipid class in surfactant, phosphatidylglycerol. Several investigators^{6,7,17} have speculated that glycogen depletion and surfactant production are related and that glycogen breakdown could supply the glycerol and fatty acid precursors required for synthesis of surfactant phospholipids.

Recently, the involvement of PAF in the stimulation of glycogenolysis was suggested by Shukla et al.,⁴ who demonstrated that PAF (2×10^{-10} mol/L) caused a threefold increase in glucose release from perfused rat livers within 2 minutes. The mechanism of the PAF stimulation of liver glycogenolysis is unknown although it appears to be similar but not identical⁴ to that of vasopressin, angiotensin II, and α -adrenergic agonists, which mediate glycogenolysis by an elevation in the concentration of cytosolic ionized calcium.¹⁸ We propose that the elevated concentration of PAF in fetal lung may be the event initiating glycogenolysis, which subsequently triggers a cascade of reactions leading to the formation of surfactant phospholipids.

The concentrations of the PAF lipid precursors lyso-PAF and 1-alkyl-2-acyl-glycero-3-phosphocholine present in the lung may be sufficient to support synthesis of PAF (Table I). We have also demonstrated that the activity of acetyltransferase increases as lung maturation progresses (Fig. 2) and that the concentration of PAF is elevated during this period of development (Fig. 4). The reciprocal relationship between the levels of glycogen and PAF in the lung explants is consistent with the view that PAF may induce glycogenolysis. In contrast to that in the liver, glycogen breakdown in lung tissue should result in increased pyruvate formation

since this tissue contains a relatively low activity of glucose-6-phosphatase.¹⁹ The increased glycolytic rate could provide the glycerol backbone for phospholipid biosynthesis as well as elevating the level of acetylcoenzyme A, which, in turn, is utilized for fatty acid biosynthesis. Ultimately, the glycerol and fatty acid moieties derived from glycogen result in the increased rate of surfactant biosynthesis that occurs during fetal lung development. The increase in acetylcoenzyme A concentration may also serve to further increase PAF biosynthesis, thus creating a cyclic process to perpetuate glycogenolysis. PAF synthesized in the type II cell could then be secreted in association with surfactant.

In most species the capacity to synthesize surfactant in the fetal lung is the last major system to develop. We²⁰ have suggested that the fetus provides the signal(s) for the initiation of parturition. The signal is translocated to the amnion tissue via the amniotic fluid. We have previously reported that PAF is present only in the amniotic fluid obtained from women at term and in labor.¹ These observations have recently been confirmed and extended.² We have also demonstrated that PAF can stimulate prostaglandin E₂ formation in amnion tissue.¹⁴ Thus PAF synthesized in the lung in concert with fetal lung maturation may be associated with the lamellar bodies¹ and be secreted by the type II pneumonocyte into the amniotic fluid where it interacts with amnion tissue to facilitate the initiation of parturition.

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Sequelae of induced first-trimester abortion

A prospective study assessing the role of postabortal pelvic inflammatory disease and prophylactic antibiotics

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A follow-up study was undertaken in 493 women who had participated in a clinical, controlled trial with the object of assessing the role of postabortal pelvic inflammatory disease and prophylactic antibiotics in the development of sequelae. Information about dysmenorrhea, dyspareunia, chronic pelvic pain, episodes of pelvic inflammatory disease, infertility, births, induced and spontaneous abortions, and ectopic pregnancies were obtained from 382 of the women and from 38 of 40 women who had contracted postabortal pelvic inflammatory disease during the previous study. Significantly elevated rates in women with postabortal pelvic inflammatory disease compared with women without this disease were found for spontaneous abortion (22% versus 5%, $p < 0.0005$), secondary infertility (10% versus 2%, $p < 0.05$), dyspareunia (20% versus 5%, $p < 0.005$), and chronic pelvic pain (14% versus 2%, $p < 0.001$). Nonsignificant differences were observed for ectopic pregnancy and dysmenorrhea. A new episode of pelvic inflammatory disease within the first year after abortion was observed more often in women with postabortal pelvic inflammatory disease than in women without infection (41% versus 5%, $p < 0.0001$). Prophylactic antibiotics decreased the rates of spontaneous abortion and dyspareunia ($p < 0.05$ in both instances). (AM J OBSTET GYNECOL 1986;155:76-80.)

Key words: Postabortal pelvic inflammatory disease, induced first-trimester abortion, induced abortion sequelae, prophylactic antibiotics

Induced first-trimester abortions are often performed in young healthy women who intend later to bear a child. Postabortal infectious complications with subsequent tubal damage may affect the fecundity of these women in the forms of ectopic pregnancy, infertility, and spontaneous abortion. In addition, postabortal pelvic inflammatory disease may result in adhesions and fibrous tissue causing dysmenorrhea, dyspareunia, and chronic pelvic pain.

Several studies have assessed the risks of spontaneous abortion, secondary infertility, and ectopic pregnancy after induced abortion.¹⁻¹⁵ Most of these have been retrospective case-control studies and have not focused on women with postabortal infectious complications. Limited information about dysmenorrhea, dyspareunia, and chronic pelvic pain as possible sequelae to postabortal pelvic inflammatory disease is available.

The present study has included follow-up of a population of women who underwent first-trimester abor-

tion in a clinical, controlled trial testing the effect of prophylactic antibiotics on postabortal infection. The object of the present study has been to compare the frequencies of spontaneous abortion, infertility, ectopic pregnancy, dysmenorrhea, dyspareunia, chronic pelvic pain, and recurrent pelvic inflammatory disease in women with postabortal pelvic inflammatory disease with those in women without the disease. The influence of prophylactic antibiotics on these sequelae was also examined.

Material and methods

The patients included in the present study comprised all 493 women who had participated in a clinical, controlled trial in 1978 and 1979.¹⁶ In the trial 254 women received prophylactic penicillin G, 2 million IU intramuscularly, one half hour before and 3 hours after first-trimester abortion followed by oral pivampicillin, 350 mg for 4 days. Two hundred thirty-nine received similar placebo medication.

Before the abortion, information about previous births, spontaneous and induced abortions, episodes of pelvic inflammatory disease, and the occurrence of dysmenorrhea, dyspareunia, and chronic pelvic pain was obtained. The women's socioeconomic data were used

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Table I. Association between postabortal pelvic inflammatory disease and spontaneous abortion and the influence of prophylactic antibiotics

	<i>Postabortal PID</i>	<i>No postabortal PID</i>	<i>Total</i>	χ^2 Test
Spontaneous abortion	7	15	22	$p < 0.0005$
No spontaneous abortion	25	278	303	
Total	32	293	325*	
Antibiotic group	1	9	10	$p < 0.05$
Placebo group	6	6	12	
Total	7	15	22	

PID = Pelvic inflammatory disease.

*Question not answered by 57 women.

to place them in one of five social classes. Gestational age and the application of an intrauterine contraceptive device were noted.

All women included in the trial were seen 4 weeks after the abortion when a pelvic examination was performed. Forty women were found to have postabortal pelvic inflammatory disease according to the criteria of infection used in the study. Fourteen of these (5.5%) were in the treatment group and 26 (10.9%) in the placebo group ($p = 0.05$). The difference could be attributed to a selective prophylactic effect in women with a history of pelvic inflammatory disease. The rate of infection in this group was 22.4% among those receiving placebo and 2.1% among those receiving antibiotics ($p = 0.006$).

During September, 1984, these 493 women all received a questionnaire. The women were asked to answer yes or no to the occurrence of dysmenorrhea, dyspareunia, chronic pelvic pain, episodes of pelvic inflammatory disease, infertility, births, induced and spontaneous abortions, ectopic pregnancies since 1978, and the current use of hormonal contraception or an intrauterine contraceptive device. Any affirmative answer required that the time of occurrence be stated. Supplementary information was collected from hospital records and the women's physicians.

Only dysmenorrhea, dyspareunia, and chronic pelvic pain that had started in association with the induced abortion in 1978 to 1979 were registered as sequelae, that is, women who also had had these complaints before the abortion were excluded. Episodes of pelvic inflammatory disease were registered as sequelae if they had occurred during the first year after the abortion. Infertility was registered when a wanted pregnancy had not occurred within 1 year. Information regarding the sperm quality of the male partner was obtained. All ectopic pregnancies were confirmed through hospital records.

Table II. Association between postabortal pelvic inflammatory disease and secondary infertility and the influence of prophylactic antibiotics

	<i>Postabortal PID</i>	<i>No postabortal PID</i>	<i>Total</i>	χ^2 Test
Secondary infertility	3	6	9	$p < 0.005$
No secondary infertility	28	317	345	
Total	31	323	354*	
Antibiotic group	0	3	3	$p < 0.2$
Placebo group	3	3	6	
Total	3	6	9	

PID = Pelvic inflammatory disease.

*Question not answered by 28 women.

The regional scientific-ethical committee approved the study and the questionnaire, which did not refer to the abortion performed in 1978 or 1979.

Results

Information was obtained from 382 (77%) of the 493 women included in the original study and from 38 of the 40 women (95%) who had a postabortal infection in 1978 or 1979. No one had died, but two had emigrated.

The association between postabortal pelvic inflammatory disease and subsequent spontaneous abortion is shown in Table I. The rate of spontaneous abortion after postabortal pelvic inflammatory disease was 22% versus 5% after an induced abortion not complicated by pelvic inflammatory disease ($p < 0.0005$). There was a significant association between the use of prophylaxis and a lower rate of spontaneous abortion (Table I).

Secondary infertility proved to be associated with the occurrence of postabortal pelvic inflammatory disease (Table II). Three of 31 women (9.7%) with postabortal pelvic inflammatory disease and six of 323 (1.9%) without the disease reported infertility lasting more than 1 year. Three of the women without postabortal pelvic inflammatory disease did become pregnant, two after 1½ and 2 years, respectively, and one after a tubal surgical procedure. Two women were not included in the follow-up because of male infertility.

Prophylaxis did not influence the frequency of secondary infertility (Table II).

Five of 323 (1.5%) women reported ectopic pregnancy. All occurred in women without postabortal pelvic inflammatory disease, the difference between rates in women with and in those without postabortal pelvic inflammatory disease being nonsignificant ($p = 0.5$). Three had received prophylaxis and two placebo.

Table III. Association of postabortal pelvic inflammatory disease and dyspareunia and the influence of prophylactic antibiotics

	<i>Postabortal PID</i>	<i>No postabortal PID</i>	<i>Total</i>	χ^2 Test
Dyspareunia	6	15	21	$p < 0.005$
No dyspareunia	24	293	317	
Total	30	308	338*	
Antibiotic group	0	7	7	$p < 0.05$
Placebo group	6	8	14	
Total	6	15	21	

PID = Pelvic inflammatory disease.

*Question not answered by 44 women.

Table IV. Association of postabortal pelvic inflammatory disease and chronic pelvic pain and the influence of prophylactic antibiotics

	<i>Postabortal PID</i>	<i>No postabortal PID</i>	<i>Total</i>	χ^2 Test
Chronic pelvic pain	4	7	11	$p < 0.001$
No chronic pelvic pain	25	316	341	
Total	29	323	352*	
Antibiotic group	1	4	5	$p < 0.9$
Placebo group	3	3	6	
Total	4	7	11	

PID = Pelvic inflammatory disease.

*Question not answered by 30 women.

Dysmenorrhea after induced abortion was observed, with nonsignificant differences between women with postabortal pelvic inflammatory disease and women without this complication.

The occurrence of dyspareunia was highly correlated with postabortal pelvic inflammatory disease (Table III). Twenty percent with postabortal pelvic inflammatory disease complained of dyspareunia after this occurrence, compared with 4.8% without postabortal pelvic inflammatory disease ($p < 0.005$).

A significant association between the administration of prophylactic antibiotics and a reduced frequency of dyspareunia was observed (Table III).

Chronic pelvic pain was reported significantly more often in women with postabortal pelvic inflammatory disease than in women without, 13.8% versus 2.2% ($p < 0.001$, Table IV).

No significant association between the administration of prophylaxis and the rate of chronic pelvic pain was observed (Table IV).

Recurrent episodes of pelvic inflammatory disease, that is, a new episode of pelvic inflammatory disease within the first year after the abortion, was significantly

Table V. Association of postabortal pelvic inflammatory disease and recurrent episodes during first year after abortion and the influence of prophylactic antibiotics

	<i>Postabortal PID</i>	<i>No postabortal PID</i>	<i>Total</i>	χ^2 Test
Recurrent PID	11	15	26	$p < 0.0001$
No recurrent PID	16	284	300	
Total	27	299	326*	
Antibiotic group	3	5	8	$p < 0.9$
Placebo group	8	10	18	
Total	11	15	26	

PID = Pelvic inflammatory disease.

*Question not answered by 56 women.

correlated with the occurrence of postabortal pelvic inflammatory disease (Table V). Forty-one percent of women with postabortal pelvic inflammatory disease had a recurrent episode of pelvic inflammatory disease, compared with 5% of women without postabortal infection ($p < 0.0001$).

The use of prophylaxis did not significantly influence the frequency of recurrent pelvic inflammatory disease (Table V).

Comment

Studies on the effects of induced abortion on subsequent reproduction have been thoroughly reviewed.¹⁷ It is claimed that prospective abortion cohort studies, in contrast to pregnancy cohort or case-control studies, are able to calculate the absolute risks of the development of sequelae after induced abortion. Furthermore, it is stated that further studies need to focus on the female population suffering infectious complications after induced abortion. The present study was designed to fulfill these two important conditions.

The risk of spontaneous abortion after induced abortion has been assessed in several studies.¹⁰⁻¹² We found an overall risk of spontaneous abortion to be 6.8% (22 of 325, Table I). The rates of spontaneous abortion after an induced abortion have been demonstrated to be from 12% to 15% in women who later became pregnant. In contrast, the present study did not investigate how many women actually tried to become pregnant, which may explain our lower rate of 6.8%. To our knowledge no study has investigated the frequency of spontaneous abortion in women whose induced abortion was complicated by infection. A rate of 22% in such women is substantially higher than that observed in the aforementioned three studies. Future investigations will need to confirm whether postabortal pelvic inflammatory disease increases the risk of spontaneous abortion.

Secondary infertility has generally not been associ-

ated with prior induced abortion.^{4, 15} However, if the induced abortion had been complicated with pelvic inflammatory disease⁹ or was illegal,² a reduction in fertility could be demonstrated. The actual risk of secondary infertility could not be established in either study because of the design. One study in which antibiotics were given to all women undergoing abortion and with follow-up 2 years later demonstrated that 7.7% had tried to initiate a new, wished-for pregnancy for more than 6 months without succeeding. The study did include second-trimester abortions, but the distribution of gestational age groups was not reported.¹⁴ As the number of women in the present study reporting infertility after postabortal pelvic inflammatory disease is small, the risk of 10%, although significantly higher than in women without postabortal pelvic inflammatory disease, must be regarded with caution.

The relative risk of ectopic pregnancy associated with induced abortion has been reported not to be increased.^{1, 9, 15} However, when the abortion was complicated by infection, an increased risk of ectopic pregnancy could be demonstrated.⁶⁻⁸ This is in contrast to the findings in the present study where all ectopic pregnancies occurred in women without postabortal pelvic inflammatory disease. However, the number in the present study is small and prospective long-term studies including larger abortion cohorts could clarify this point.

Information about pain as a delayed complication of postabortal pelvic inflammatory disease is limited. It is generally accepted that pelvic inflammatory disease may give rise to pelvic adhesions and subsequent pain in the affected organs. It is not surprising that postabortal pelvic inflammatory disease apparently had a similar effect. The frequencies of dyspareunia and chronic pelvic pain of 20% and 14%, respectively, subsequent to postabortal pelvic inflammatory disease correspond well with the rate of 18% low abdominal pain reported after salpingitis verified by laparoscopy.¹⁸

The consequences of recurrent episodes of pelvic inflammatory disease are serious as tubal damage after one infection was observed in 13% of women, after two in 35%, and after three in 75%.¹⁸ Likewise, the risk of contracting postabortal pelvic inflammatory disease after previous episodes of pelvic inflammatory disease was 22%.¹⁶ These observations indicate that there are no doubt women who are at greater risk of suffering from pelvic inflammatory disease and its sequelae and they should be informed of this with special reference to choice of contraception, pregnancy planning, and sexual habits.

In the present study antibiotic prophylaxis had an effect on some sequelae of postabortal pelvic inflammatory disease. The numbers probably were too small to show significant differences after cross tabulation for

treatment. However, it has been demonstrated that by applying prophylaxis to the 20% of women with a history of pelvic inflammatory disease, the total number of postabortal infections could be reduced by 50%.¹⁶ Consequently, prophylaxis would reduce the frequency of sequelae.

In a randomized study, confounding variables should be equally distributed in the treatment and placebo groups, and the randomization should ensure that sequelae were almost exclusively related to the presence or absence of postabortal pelvic inflammatory disease. However, cigarette smoking was not included as a variable in the present study.

The reason that many previous studies have been unable to demonstrate any harmful effect of induced abortion can be explained by the inclusion of all induced abortions. Thereby uncomplicated abortions, which almost certainly are harmless and which constitute by far the greatest number, have masked the harmful effects of complicated abortions.

It is important to emphasize that although the frequencies of sequelae may seem high, they were high only in women with postabortal infection. These were only 10% of the total abortion population, that is, dyspareunia as a consequence of the procedure will occur in $\frac{1}{10} \times \frac{1}{5}$ or 2% of all women undergoing induced abortion.

The present study demonstrated that women with postabortal pelvic inflammatory disease had significantly higher rates of sequelae than women without infection after the induced abortion. These sequelae comprised spontaneous abortion, secondary infertility, dyspareunia, and chronic pelvic pain. Future larger studies will need to focus on women with postabortal complications to assess their risk of the development of long-term sequelae and the role of prophylactic antibiotics.

Løvens Kemiske Fabrik, Leo, Denmark, kindly performed the computational treatment of the data.

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Predicting postnatal emotional adjustment with psychosocial and hormonal measures in early pregnancy

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This prospective study showed that scores on a socioenvironmental stress questionnaire were related to numbers of crying spells and to estradiol levels in pregnancy. Estradiol levels in turn were correlated with numbers of antenatal and postnatal crying spells. Stress, hormonal response in pregnancy, and crying during pregnancy each contributed independently to predicting postpartum emotional adjustment. (*AM J OBSTET GYNECOL* 1986;155:80-2.)

Key words: Predicting postnatal emotions, antenatal stress, pregnancy hormones, crying spells

Clinical researchers have speculated for decades about possible relationships between psychosocial, hormonal, and environmental factors in relation to postnatal emotional reactions. Karnosh and Hope,¹ in 1937, suggested that postpartum psychiatric symptoms were related to hormonal changes. Billig and Bradley,² in 1946, found that progesterone alone did not help patients with postpartum emotional disorders but did prevent relapses. Hamilton³ and Larsen et al.⁴ have suggested that adrenal corticosteroids and thyroid hormones may play a part.

Other authors⁵⁻¹¹ have suggested relationships between psychophysiologic and environmental factors with postnatal emotional adjustment. Nott et al.¹² found

Table I. Intercorrelations between postnatal measures

First trimester	First trimester, PCry 1 (n = 16)	Second trimester	
		PSymp 2 (n = 13)	PCry 2 (n = 13)
PSymp 1 (n = 16)	0.62	0.74	0.61
PCry 1 (n = 16)		—	0.64

PSymp 1 = Symptom scale score, first postnatal trimester; PCry 1 = number of crying spells, first postnatal trimester; PSymp 2 = symptom scale score, second postnatal trimester; PCry 2 = number of crying spells, second postnatal trimester. All correlations were significant at the 0.01 level or greater.

that among the 30 possible correlations between biochemical variables and emotional reactions in pregnancy that they examined, four reached a level of significance: Predelivery estrogen levels tended to be higher in those women who were more irritable ($r = 0.36$, $p < 0.05$). The greater the progesterone drop, the more likely the subjects tended to be depressed within the first 10 days after delivery ($r = 0.35$,

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Table II. Correlations among antenatal and postnatal measures of stress, hormones, crying, and emotional reaction

Measure	Antenatal trimester			Postnatal trimester			
	First, ASymp 1 (n = 16)	Third		First		Second	
		Estr 3 (n = 13)	ACry 3 (n = 16)	PSymp 1 (n = 40)	PCry 1 (n = 16)	PSymp 2 (n = 13)	PCry 2 (n = 16)
First antenatal trimester							
Stress Q (n = 40)	0.69*	-0.55†	—	0.49*	—	—	—
ASymp 1 (n = 16)	—	—	0.61‡	0.58‡	0.56‡	0.64‡	0.53†
Second antenatal trimester							
Estr 2 (n = 16)	—	0.65‡	—	—	—	—	—
ACry 2 (n = 16)	—	-0.57†	0.53†	—	—	—	—
Third antenatal trimester							
Estr 3 (n = 13)	—	—	-0.67‡	—	—	—	-0.47§
ACry 3 (n = 16)	—	—	—	0.67*	0.72	—	0.76

Stress Q = Antenatal stress questionnaire; ASymp 1 = antenatal symptom scale; Estr 2 = estriol level, second antenatal trimester; ACry 2 = number of crying spells, second antenatal trimester; Estr 3 = estriol level, third antenatal trimester; ACry 3 = number of crying spells, third antenatal trimester; PSymp 1 = symptom scale score, first postnatal trimester; PSymp 2 = symptom scale score, second postnatal trimester; PCry 2 = number of crying spells, second postnatal trimester. Only correlations of 0.47 or better are shown.

*p = 0.01.

†p = 0.05.

‡p = 0.025.

§p = 0.10.

||p = 0.001.

p < 0.49), but the less likely they were to report sleep disturbance ($r = 0.49$, $p < 0.05$); the lower the estrogen levels post partum, the more sleep disturbance was reported ($r = 0.43$), $p < 0.05$). Finally, Treadway et al.¹³ reported a significant correlation of 0.76 between norepinephrine levels and depression scores.

In this article we report on the interactions between three types of variables: (1) psychological, (2) hormonal, and (3) social environmental. It is hypothesized that social environmental stresses will affect hormonal and psychological functioning; in turn these latter will be correlated with antenatal behavior and emotional response. It is also proposed that antenatal psychophysiological variables will predict postnatal emotional and behavioral responses.

Method

Subjects. Candidates for this study were 40 pregnant, mostly primiparous women who in their first antenatal trimester filled in a maternal stress questionnaire¹⁴ and a psychiatric symptom scale.¹⁵ Twenty of these women were selected to provide subjects with a full range of low, middle level, and high stress scores. Four of these 20 women, however, three with high scores and one with a moderate score, aborted spontaneously and therefore did not complete the study.

Sixteen women supplied "crying spell" data throughout the year of the study. All 40 women were aged 19 to 29. One was unmarried, another was a married Oriental, and the remainder were white, married, middle-

class, American housewives of Anglo-Saxon or North European descent.

All 40 subjects filled in the psychiatric symptom scale 3 months after delivery (see below). Those 16 who were recording their crying spells provided data for the 6-month postnatal period of the study. Estriol levels were obtained for 16 of the women in the second antenatal trimester and for 13 in the third trimester.

Antenatal measures. Six antenatal measures were used, each of which provided a score for predicting postnatal emotional reaction. These included the following variables: (1) the antenatal maternity stress questionnaire score, (2) the antenatal symptom scale score, (3) the numbers of crying spells in the second antenatal trimester (the subjects recorded every occasion in which they cried for any reason during this and subsequent periods of the experiment), (4) the second-trimester estriol level, (5) the numbers of crying spells in the third trimester, and (6) the third-trimester estriol level.

The maternity stress questionnaire has been shown to correlate significantly with the emotional outcome of pregnancy.^{5,14} The psychiatric symptom scale was adapted from the scale of Leighton et al.¹⁵

Estriol plays a role in the growth of the placenta and fetus; a drop in level signifies that the fetus is in jeopardy. Estriol level was measured with a method developed and performed during the study by Dr. M. W. Heine, with the use of electron capture and flame ionization detectors in a gas-liquid chromatography system.

Postnatal measures. The social psychiatric outcome of pregnancy was measured at 3 and 6 months post partum by the psychiatric symptom scale scores and numbers of crying spells. At 3 months all 40 women recorded their numbers of crying spells. At 6 months post partum symptom scores and crying spells were again assessed. Intercorrelations of the postnatal measures are shown in Table I.

Statistical analysis. Product-moment correlations were obtained with the continuous variables. Multiple correlations were calculated with the programs of the Statistical Package for the Social Sciences. To measure the reliability of the estriol measurements, estriol levels in two consecutive samples were correlated with each other. The reliability coefficients ranged from 0.55 to 0.78.

Results

The correlations among the antenatal and postnatal measures are shown in Table II. Only those with a coefficient >0.40 are presented.

First-trimester antenatal predictors

Stress questionnaire. Table II shows that maternal stress questionnaire scores are highly correlated with both first-trimester antenatal symptom and postnatal symptom scores. Maternal stress questionnaire scores correlated negatively with third antenatal trimester estriol levels.

Second-trimester antenatal predictors

Crying. Second antenatal trimester crying predicted third-trimester estriol levels and numbers of crying spells in the third antenatal trimester.

Third antenatal trimester predictors

Estriol. Estriol levels were highly inversely correlated with numbers of third antenatal trimester crying spells. They also predicted crying in the second postnatal trimester.

Crying. The numbers of third antenatal trimester crying spells predicted numbers of crying spells with high and very significant correlations in the first and second postnatal trimesters and psychiatric symptom scores in the first postnatal trimester. In general, if a woman reported more than 15 crying spells in any 60 day period during pregnancy, she was liable to have emotional troubles during the third antenatal trimester as well as post partum.

Multiple correlations. The multiple correlation between antenatal scores on the stress questionnaire, estriol levels, and numbers of antenatal crying spells with the postnatal symptom scale score was 0.96.

Comment

The most important findings of this study are as follows.

Crying. Regardless of its cause, crying during pregnancy by itself provided a useful clue to the patient's perinatal emotional state. We suggest that physicians treating pregnant women ask their patients to keep a daily log of numbers of crying spells. Although we cannot generalize to populations from different cultures, where crying may be more or less acceptable among women, we can offer a preliminary rule of thumb: Consider 15 or more episodes of crying in any 60-day period as a warning signal of possible emotional complications during or after pregnancy.

Socioenvironmental stress. Socioenvironmental stress measured by questionnaire in the first trimester of pregnancy predicted hormonal state during pregnancy and psychiatric symptoms after delivery. High stress was also associated with spontaneous abortion.

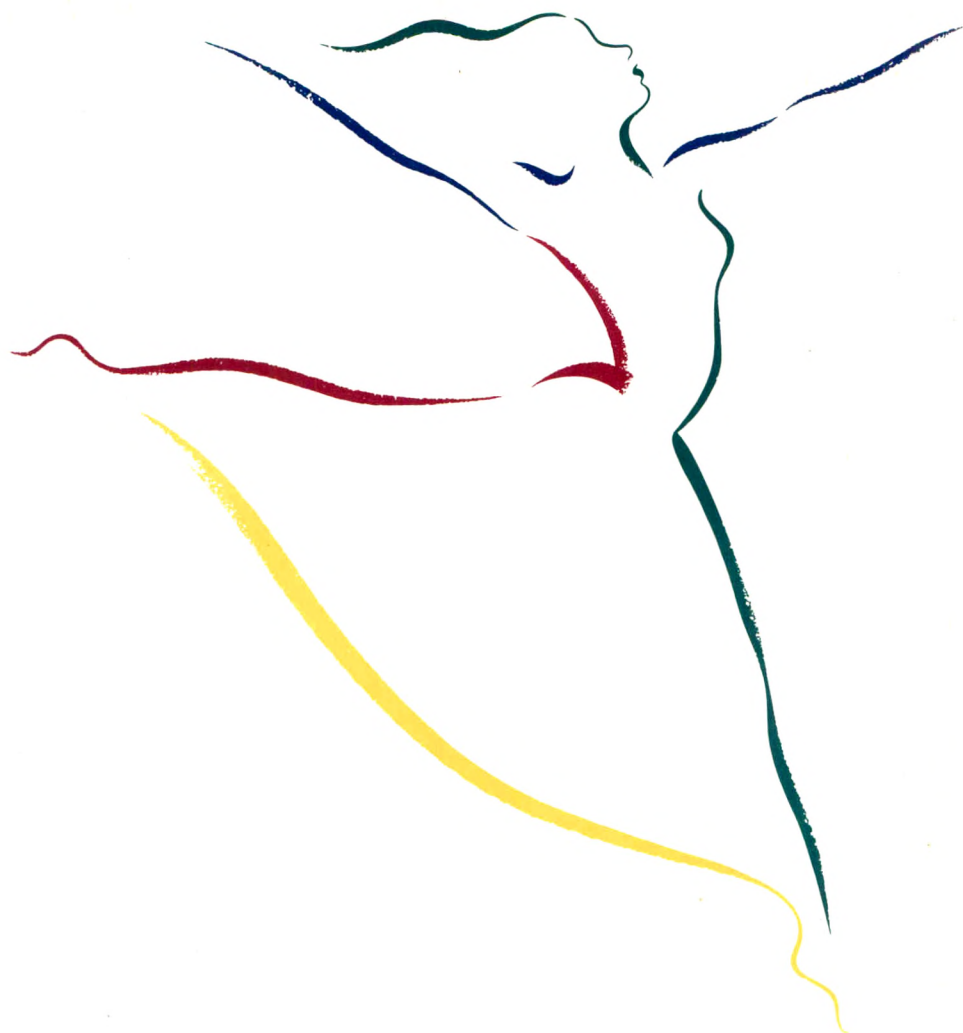
Psychiatric symptomatology. The psychiatric symptom scale administered in the first trimester of pregnancy predicted numbers of crying spells in the third antenatal trimester and in both postnatal trimesters.

Estriol. Estriol level tended to be lower in subjects who were leading stressful lives, in those who cried a great deal during and after pregnancy, and in those who underwent spontaneous abortions.

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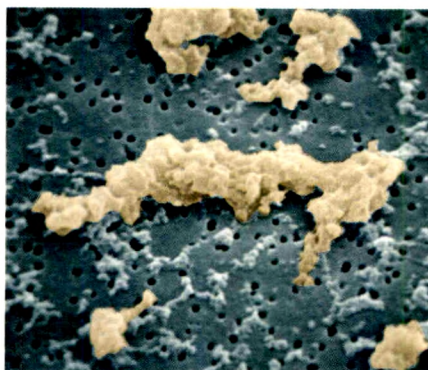
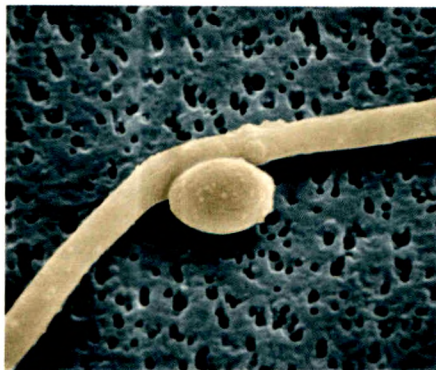
1. According to a 12-month nationwide industry audit (ending October 1985), comprised of approximately 2,000 pharmacies. Vulvar burning occurred in 2.3% and itching in 0.9% of patients on Femstat.

2. Bradbeer CS, Mayhew SR, Barlow D: Butoconazole and miconazole in treating vaginal candidiasis. *Genitourinary Med* 61:270-272, 1985. (Study conducted in non-pregnant patients.)

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Scanning electron photomicrographs (5000X) of *Candida albicans* grown for 48 hours in serum (left), and in serum with 200 mcg/ml butoconazole nitrate (right).

Brief Summary

INDICATIONS:

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CONTRAINDICATIONS:

FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

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If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient:

Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis:

Animal studies have not been done.

Mutagenesis:

Mutagenicity studies were negative.

Impairment of Fertility:

Animal studies showed no impairment of fertility.

Pregnancy Category C:

Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:

Use with caution.

Pediatric Use:

Safety and efficacy have not been established.

ADVERSE REACTIONS:

Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:

Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:

Federal law prohibits dispensing without prescription.

December 1985



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Prognostic value of peritoneal washings in patients with malignant mixed müllerian tumors of the uterus

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Peritoneal washings from 36 women with all pathologic stages of mixed müllerian tumors of the uterus (27 homologous stroma, nine heterologous stroma) were reviewed in a blinded retrospective fashion for the presence and type of malignant cells collected. Malignant tumor cells were demonstrated in the washings of 16 patients; 13 contained adenocarcinoma only, two contained adenocarcinoma and sarcoma, and one contained sarcoma cells only. Cox's logistic regression analysis showed that cytologic examination of peritoneal washings when combined with pathologic staging provides a statistically significant discriminant of disease-free survival. Patients with a favorable prognosis (pathologic Stage I and peritoneal washings free of malignancy) had sevenfold increased disease-free survival compared with survival of the patients with an unfavorable prognosis (pathologic Stages II, III, or IV or malignant cells present in peritoneal washings). Three of the 18 patients with pathologic Stage I disease manifested malignant tumor cells in the peritoneal washings and all three patients died of disease in less than 1 year. The presence of malignant cells in peritoneal washings in patients with mixed müllerian tumor apparently limited to the uterus suggests a clinical course that is similar to that of those with more advanced disease. (AM J OBSTET GYNECOL 1986;155:83-9.)

Key words: Peritoneal washings, mixed müllerian tumors

Peritoneal washings are valuable prognostic indicators in ovarian carcinoma¹ and are suggested to be important in endometrial carcinoma^{2,3}; their role in carcinoma of the cervix remains questionable.⁴ The information obtained from the washings is used to plan adjuvant therapy for those patients with cytologic evidence of malignant cells in the peritoneal cavity. A role for peritoneal washings in the evaluation of patients with malignant mixed müllerian tumors of the uterus has not been established to date. This study was undertaken to determine the significance of peritoneal washings in the evaluation of patients with mixed müllerian tumors of the uterus.

Material and methods

The files and surgical pathologic material from 41 patients with mixed müllerian tumor treated at Duke University Medical Center from 1974 and 1985 were reviewed. Four patients were excluded because of previous partial resection of disease (one patient) or lack

of postoperative clinical care and absence of any information regarding their subsequent course (three patients). One of the patients had clinical evidence of ascites at the time of evaluation and is addressed separately. Thus the final study group consisted of 36 patients without clinical evidence of ascites.

All patients were assessed preoperatively by a differential cervical dilatation and curettage of the endometrium and endocervix. All patients underwent peritoneal washings, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and selective pelvic and para-aortic lymphadenectomy at the time of the initial surgical extirpation. Tumor presence, histologic type, depth of myometrial invasion, and lymph node involvement were assessed in the surgical specimen. The number of sections of tumor and myometrium taken for histologic study varied from three to 20. The tissue was fixed in buffered formalin, routinely treated in serial alcohol dilutions, and embedded in Paraplast (Monoject Scientific, St. Louis, Missouri). The tissue was sectioned at 5 m μ and stained with hematoxylin and eosin. All tumors were verified to have both malignant epithelial and stromal components.

The peritoneal washings were obtained from all patients at the time of the initial surgical extirpation. As soon as the peritoneal cavity was opened, approximately 100 ml of sterile balanced salt solution was instilled into the pelvis, over the uterus, fallopian tubes, and ovaries, and allowed to collect in the cul-de-sac.

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Table I. Clinical outcome of patients with pathologic Stage I mixed müllerian tumor of the uterus as reflected by depth of myometrial invasion

Patient No.	Malignant stromal component	Cytologic results of peritoneal washing	Therapy	Clinical status
<i>Tumor confined to superficial endometrium</i>				
1	Homologous	Negative	None	NED, 84 mo
2	Homologous	Negative	None	NED, 100 mo
3	Homologous	Negative	None	NED, 44 mo
4	Homologous	Negative	A, V, Dact., C	R, 12 mo; DOD ²
<i>Tumor invasive to 1/3 myometrial thickness</i>				
5	Homologous	Negative	A	NED, 79 mo
6	Homologous	Negative	5000 rad	NED, 84 mo
7	Homologous	Negative	V, Dact., C	R, 21 mo
8	Homologous	Negative	V, Dact., C	R, 31 mo
9	Heterologous	Negative	V, Dact., C	R, 29 mo
10	Homologous	Adenocarcinoma	P ³²	R, 7 mo; DOD
11	Homologous	Adenocarcinoma	V, Dact., C	R, 3 mo; DOD
<i>Tumor invasive to >1/3 myometrial thickness</i>				
12	Homologous	Negative	V, Dact., C	NED, 84 mo
13	Heterologous	Negative	V, Dact., C	NED, 44 mo
14	Heterologous	Negative	V, Dact., C	R, 12 mo
15	Heterologous	Negative	V, Dact., C	NED, 25 mo
16	Homologous	Negative	5000 rads	NED, 24 mo
17	Homologous	Adenocarcinoma, Sarcoma	V, Dact., C	R, 12 mo; DOD
18	Homologous	Negative	Refused treatment	R, 9 mo; DOD

NED = No evidence of disease; DOD = dead of disease; R = recurrent disease; A = doxorubicin; V = vincristine; Dact. = dactinomycin; C = cyclophosphamide; P³² = intraperitoneal chromic phosphate P 32 suspension.

The fluid was removed and sent immediately for analysis. Membrane filter preparations and smears were fixed in 95% ethyl alcohol and stained by a modified Papanicolaou technique. All preparations of surgical and cytologic materials were reviewed by two examiners without prior knowledge of the patient's treatment or course.

In the 36 patients without ascites, initial staging was by the clinical parameters adapted by the International Federation of Gynecology and Obstetrics, in 1970, for carcinoma of the endometrium. After evaluation of the surgical pathologic findings the patients underwent re-staging of disease to reflect the documented postoperative extent of disease. Chemotherapy and/or radiotherapy were administered on the basis of the depth of myometrial invasion, lymph node involvement, presence or absence of malignant cells in the peritoneal washings, and the clinical status of the patient.

Special statistical methods. Cox's proportional hazards regression model for censored survival data was used to identify prognostic factors in the study. A multivariate logistic regression model was fitted to the data of the 36 patients to determine the variables related to disease-free survival as measured from the time of diagnosis to disease recurrence or the end of the study (December, 1985), whichever occurred first. The prognostic variables considered were age at diagnosis, race, pathologic staging (I to IV), and cytologic evidence of malignancy (the presence or absence of malignant cells in the peritoneal washings). The method applied used

the PHGLM program of the Statistical Analysis System by F. E. Harrell, Jr.⁵ The data were examined by the backward elimination technique, which starts with the complete regression model, one that includes all independent variables, and eliminates variables singularly until all remaining variables are significant. Tests of significance are based on the assumption that twice the difference in log likelihoods has a χ^2 distribution.⁶ In addition, Kaplan-Meier disease-free survival curves were constructed for patients with "localized" versus "advanced" disease and divergence of the curves was tested for significance by log-rank tests.⁶ Those patients with "localized disease" were defined as having pathologic Stage I and no malignant cells in peritoneal washings, whereas patients with "advanced" disease were in Stages II, III, or IV or had malignant cells present in peritoneal washings. Divergence of the Kaplan-Meier disease-free survival curves for "localized" and "advanced" disease was greatest when "localized disease" was restricted to those patients with Stage I disease and no malignant cells in peritoneal washings. Also recurrence rates per patient month of follow-up for patients with pathologic Stage I disease with or without malignant cells in peritoneal washings were compared for statistical significance by two-tailed symmetric Z tests.⁷

Results

The pathologic and clinical profiles of 18 patients with pathologic Stage I mixed müllerian tumor of the uterus are presented in Table I. Fifteen of 18 of these

Table II. Clinical outcome of patients with pathologic Stages II, III, or IV mixed müllerian tumor of the uterus

Patient No.	Malignant stromal component	Cytologic results of peritoneal washing	Therapy	Clinical status
Stage II				
19	Heterologous	Adenocarcinoma	P ³²	NED, 6 mo
20	Homologous	Negative	A	R, 17 mo
21	Homologous	Adenocarcinoma	P ³² , V, Dact., C	NED, 24 mo
22	Heterologous	Adenocarcinoma	A, Dact.	R, 9 mo; DOD
23	Homologous	Adenocarcinoma	None	NED, 2 mo
Stage III				
24	Homologous	Adenocarcinoma	P ³² , A	R, 36 mo
25	Homologous	Adenocarcinoma	V, Dact., C	R, 3 mo; DOD
Stage IV				
26	Homologous	Negative	V, Dact., C	R, 24 mo; DOD
27	Homologous	Negative	V, Dact., C	R, 9 mo; DOD
28	Heterologous	Negative	V, Dact., C	R, 7 mo; DOD
29	Homologous	Negative	None	R, 6 mo; DOD
30	Heterologous	Sarcoma	1000 rad	R, 7 mo; DOD
31	Heterologous	Adenocarcinoma	V, Dact.	R, 6 mo; DOD
32	Homologous	Adenocarcinoma	A	R, 6 mo; DOD
33	Heterologous	Adenocarcinoma, Sarcoma	V, Dact., C	NED, 24 mo
34	Homologous	Adenocarcinoma	V, Dact., D, P ³²	R, 14 mo
35	Homologous	Adenocarcinoma	B, VIN, Cis. ¹¹	R, 3 mo; DOD
36	Homologous	Adenocarcinoma	A, C	R, 1 mo; DOD

B = Bleomycin; VIN = vinblastine; Cis = cisplatin. For other abbreviations see footnote to Table I.

patients had peritoneal washings with no evidence of malignancy. Of the three patients found to have malignant cells in the washings, two patients had tumor invasive to less than one third of the total myometrial thickness and one patient had tumor invasive to greater than one third of the total myometrial thickness. None of the patients with tumor confined to the endometrium had malignant cells in the peritoneal washings. Only four patients in this group were noted to have heterologous malignant stromal elements as a component of the tumor. None of those patients with heterologous malignant stromal elements were found to have malignant cells in the peritoneal washings; two patients developed recurrent disease at 12 and 29 months while two other patients remain disease-free at 25 and 44 months after operation.

It is notable that malignant cells were detected in the peritoneal washings of three of the 18 patients with pathologic Stage I disease, and all three patients developed recurrences within the first year of follow-up. By comparison, nine of the 15 patients with pathologic Stage I disease and peritoneal washings free of malignant cells were alive without evidence of disease at the end of the study. This difference in recurrence risk per month of follow-up (three recurrences in 22 months of follow-up versus six recurrences in 682 months of follow-up) approaches significance by a two-tailed symmetric Z test ($p < 0.08$).

Of the 18 patients with pathologically more advanced disease, 13 patients had malignant cells identified in the peritoneal washings (Table II). As anticipated from the

extent of the disease, 13 of the 18 patients developed recurrent disease or died of carcinomatosis within 24 months.

Fig. 1 demonstrates the disease-free survival for the entire patient study group. The median survival was 15 minutes, while the probability of long-term survival (>30 months) was 30%. Table III shows the results of Cox linear regression, which tests the association between disease-free survival and the observed prognostic indicators. When the variables were tested alone in univariate models, pathologic staging and cytologic evaluation of peritoneal washings both showed highly significant predictive values, whereas age and race did not. Results of the multivariate model, which tests all variables simultaneously, show that pathologic staging was the best singular predictor of survival ($p < 0.001$) while cytologic evaluation added secondarily only approached statistical significance ($p < 0.11$). Thus, as expected, pathologic staging and cytologic evaluation of peritoneal fluid are not independent of each other. However, it is important to note that both tests used in combination yielded a model having a highly significant statistical association with disease-free survival ($p < 0.004$). This is illustrated in Fig. 2, which contrasts the disease-free survival curves of patients with a favorable prognosis (pathologic Stage I and the absence of malignant cells in peritoneal washings) with that of patients with an unfavorable prognosis (pathologic Stage II, III, or IV or the presence of malignant cells in peritoneal washings). The curves show statistically significant divergence by a log-rank test ($p < 0.0001$) and reflect an

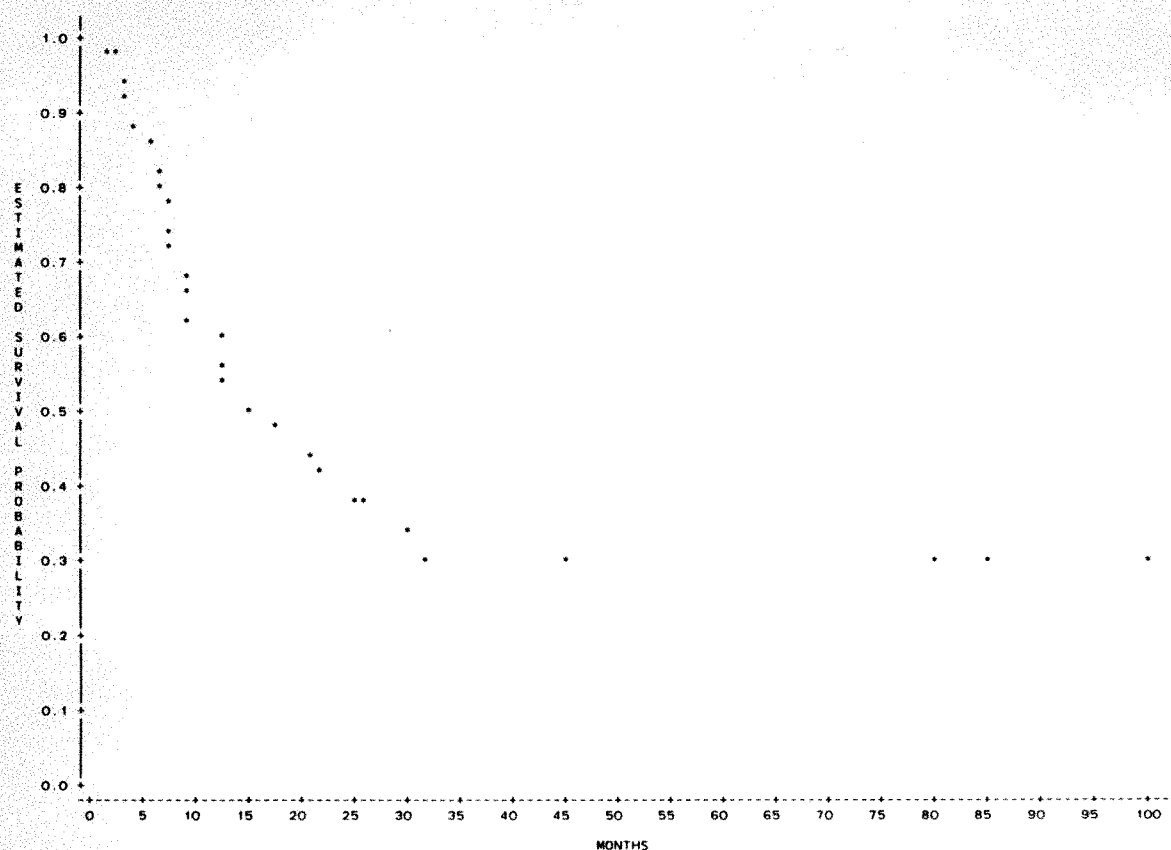


Fig. 1. Disease-free survival curve for all 36 patients with malignant mixed müllerian tumor.

Table III. Significance levels of prognostic indicators of disease-free survival in mixed müllerian tumor of the uterus based on Cox's linear regression analysis

Variable	Model tested	
	Univariate	Multivariate
Pathologic stage	0.001	0.001
Cytologic evaluation	0.003	0.11
Age	0.54	0.35
Race	0.89	0.50
Pathologic/cytologic results*	—	0.004

*A model combining pathologic staging and cytologic examination of peritoneal fluid whereby Stage I and peritoneal washings without malignant cells denote favorable prognosis and Stages II to IV or peritoneal washings with malignant cells denote unfavorable prognosis.

approximately sevenfold difference in disease-free survival at 24 months of follow-up. The probability of long-term disease-free survival (>30 months) for patients with favorable histologic and cytologic features is 60%.

Malignant cells were identified in the washings of 16 patients in the study group. Thirteen washings had adenocarcinoma only, and two had both adenocarci-

noma and sarcoma recognized, while sarcoma was the only identifiable malignant component in another peritoneal washing. Cytologically, the characteristics of adenocarcinoma included solid, tight clusters of uniform cells, with increased nuclear:cytoplasmic ratios, peripheral coarse chromatin, and prominent nucleoli (Fig. 3). These groups were easily recognized to be distinct from the sheets of reactive mesothelium frequently identified in peritoneal wash material. Sarcoma cells displayed greater variability with frequent, single multinucleate cells often with complex nuclear membrane folds and modest amounts of cytoplasm (Fig. 4). In addition, occasional single, uninucleate cells with increased nuclear:cytoplasmic ratios and coarse chromatin resembling the sarcoma cells of endometrial stromal origin were also identified. No cross striations within the cytoplasm were identified in any of the sarcoma cells.

Comment

Malignant mixed müllerian tumors are known for their early myometrial invasion and aggressive nature with dissemination of tumor throughout the abdominal cavity, lymphatic spread to lymph nodes, and hematogenous dissemination to visceral organs such as the liver, lungs, bone marrow, and vagina.⁸⁻¹¹ Reported 5-

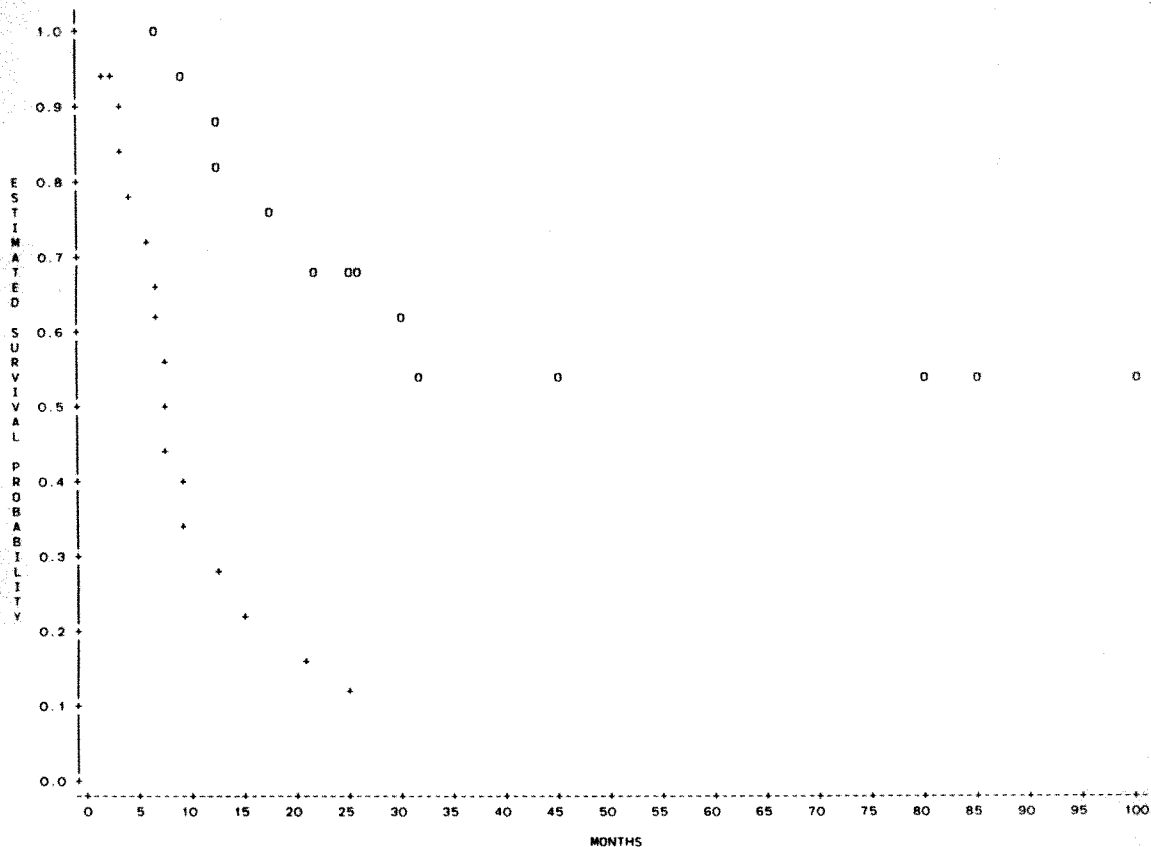


Fig. 2. Disease-free survival curves for patients with mixed müllerian tumor of the uterus. Patients with pathologic Stage I disease and peritoneal washings without malignant cells (0). Patients with pathologic Stages II, III, or IV disease or peritoneal washings with malignant cells (+).

year survival rates for this malignancy, including both malignant homologous and heterologous stroma, range from 15% to 30%.^{9, 11, 12} The significance of correct histopathologic identification is made complex by those patients who continue to have unusually long survivals as well as those whose course is abrupt. While extensive penetration of the myometrium and serosal involvement by the tumor are recognized to be poor prognostic indicators,^{8, 9, 11-13} tumor limited to a polyp may imply a longer survival.^{14, 15} Of continued debatable significance is the presence of malignant homologous versus heterologous stroma,^{9, 11} the presence or absence of rhabdomyoblasts,⁸ and the presence of chondroid differentiation.^{8, 13} The presence or absence of malignant cells in the peritoneal washings has received little attention in the assessment of these patients.

Peritoneal washings are an accepted part of the evaluation of patients with carcinoma of the ovary¹ and are routinely performed at Duke University Medical Center for the assessment of early peritoneal involvement in patients with adenocarcinoma of the endometrium.^{2, 3} The presence of malignant cells in peritoneal washings in which there is no apparent break in the capsule of an ovarian tumor, no omental implants, no

obvious involvement of the serosa of the uterus by an ovarian tumor, and no abnormal accumulation of fluids in the pelvic or peritoneal cavity has implications with regard to recurrence.¹ A similar importance for peritoneal washings in patients with adenocarcinoma of the endometrium has been suggested.^{2, 3}

The purpose of this study was to assess the significance of peritoneal washings in patients with mixed malignant müllerian tumors of the uterus. Our study demonstrates that in patients with disease limited to the uterus by pathologic staging, the cytologic presence of malignant cells in peritoneal washings portends a poor prognosis. Specifically, three such patients in our study group died of disease within 12 months. Furthermore, those without pathologic evidence of disease outside of the uterus demonstrate significantly better survival than that of those with advanced disease (Fig. 2). In spite of the observed value of these factors as discriminants for prognosis, pathologic staging combined with cytologic evaluation failed to predict recurrent disease in six patients with pathologic Stage I disease and no malignant cells in the peritoneal washings. In those patients who are discovered to have advanced disease at the time of surgical extirpation and after pathologic

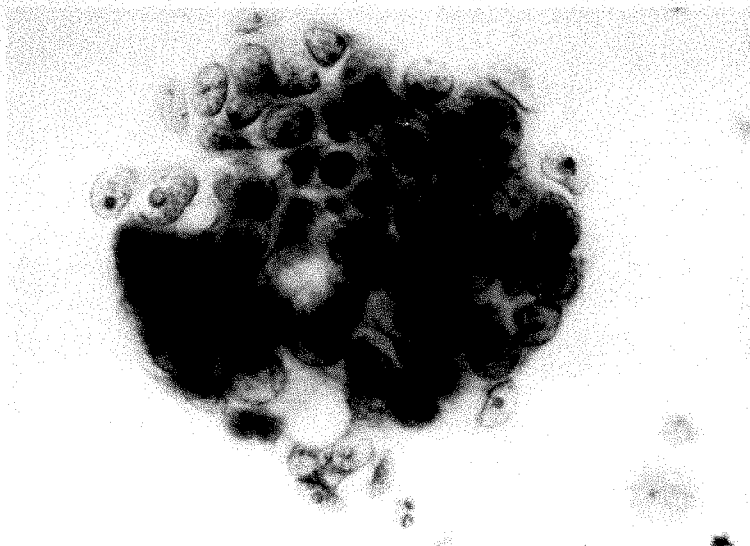


Fig. 3. A cluster of adenocarcinoma cells in peritoneal washings. (Membrane filter preparation. Modified Papanicolaou stain. Original magnification $\times 1600$.)

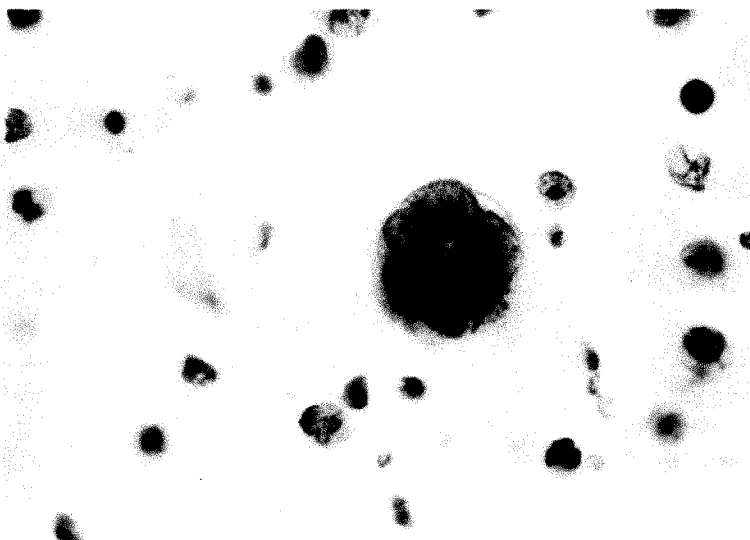


Fig. 4. A single, multinucleate undifferentiated sarcoma cell in peritoneal washing. (Membrane filter preparation. Modified Papanicolaou stain. Original magnification $\times 1600$.)

evaluation, the value of peritoneal washings appears to be questionable.

One of our patients had clinical evidence of ascites with adenocarcinoma at the time of evaluation along with gross evidence of omental involvement by metastatic tumor. An attempt was made to extirpate as much tumor as possible. She refused further chemotherapy and died of metastatic disease 10 months after operation. In this review, no patients were identified with clinical evidence of ascites but without cytologic evidence of tumor cells in the fluid. Previous observations in patients with metastatic carcinoma of the ovary and ascites but no cytologic evidence of malignancy suggested that such patients have a slightly longer survival

when compared with that of those with cytologic evidence of malignancy.¹⁶

This study supports the general concept that the extent of disease discovered at the time of surgical extirpation is an important prognostic characteristic. The number of tumors with heterologous stroma was too small to make any statement regarding the presumably poor prognosis associated with mixed müllerian tumors of this subset. This study group was too small to make any conclusions regarding the effectiveness of chemotherapy and/or radiotherapy or the prognostic significance of the presence of rhabdomyoblastic elements or chondroid differentiation.

For those patients with mixed müllerian tumor of the

uterus in whom the tumor is apparently limited to the uterus, peritoneal washings with malignant cells are indicative of early dissemination of the peritoneal cavity and portend a prognosis similar to that of those patients with advanced stages of disease. Staging by combining the cytologic interpretation of the peritoneal washing and histopathologic findings from the hysterectomy specimen and lymph node dissection is recommended to refine the prognosis and perhaps affect the selection of combined chemotherapy and radiation therapy for any given patient.

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Have Liley charts outlived their usefulness?

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London, England

Fetal blood and amniotic fluid samples were obtained fetoscopically from 59 rhesus-isoimmunized pregnancies at 18 to 25 weeks' gestation. Fetal hemoglobin was measured and amniotic fluid optical density deviation at a wavelength of 450 nm determined. Two sets of normal reference values for optical density at 450 nm and fetal hemoglobin at 16 to 36 and 16 to 25 weeks were established from 475 amniotic fluid and 153 fetal blood samples obtained from pregnancies not complicated by fetal hemolysis. As expected, there was a significant linear correlation between the degree of fetal anemia and the amniotic fluid optical density at 450 nm in rhesus-isoimmunized pregnancies. However, the values of optical density at 450 nm were widely scattered, thereby limiting their ability to predict accurately the severity of disease in these second-trimester pregnancies. In 25 of the patients, the value of optical density at 450 nm was determined at 6 to 16 days before fetoscopy. The severity of fetal anemia could not be predicted by the trend in optical density at 450 nm. These data suggest that the only reliable method to determine the severity of rhesus isoimmunization in the second trimester of pregnancy is the direct measurement of fetal hemoglobin. (AM J OBSTET GYNECOL 1986;155:90-4.)

Key words: Rhesus isoimmunization, Liley charts, fetoscopy

Although bilirubin staining of the amniotic fluid in cases of severe jaundice in the newborn infant was first described by Ballantyne as long ago as 1892, it was not until the 1950s that the association between the amount of blood pigment in the liquor amnii and the degree of erythrocyte destruction was recognized.^{1,2} Subsequently, Liley³⁻⁵ offered a systematic approach to amniotic fluid analysis in the management of rhesus-immunized pregnancies, an approach that has become the established method for assessing the severity of the disease and for timing obstetric interventions such as early delivery or intrauterine fetal blood transfusions.

In a series of 101 rhesus-immunized pregnancies, Liley³ performed amniocenteses at 27 to 41 weeks' gestation, measured spectrophotometrically the amniotic fluid bilirubin concentration by the deviation in optical density at a wavelength of 450 nm (ΔOD_{450}), and plotted the results on semilogarithmic graph paper against the gestational age. He divided the values into three prognostic zones according to the outcome of pregnancy and the severity of the disease as assessed by the hemoglobin concentration at delivery. The upper zone

included those severely affected fetuses who were at risk for intrauterine or neonatal death while the lower zone contained rhesus-negative or mildly affected rhesus-positive fetuses. In the middle zone, which was subsequently subdivided into upper and lower subzones,⁴ the prognosis was found to depend on the magnitude of the deviation as well as the trend in ΔOD_{450} , that is, whether it was rising or falling after a second or possibly third determination. It was recommended that when the ΔCD_{450} values fell in the lower zone, no intervention was needed. However, if the values appeared in the upper zone, delivery should be undertaken or, if the fetus were too premature, an intrauterine blood transfusion given.⁵

In the 1960s, other investigators confirmed and elaborated on the findings of Liley, often devising their own systems for interpreting ΔOD_{450} measurements.⁶ However, the high perinatal mortality rate at the time for premature infants of 28 to 32 weeks' gestation and the high procedure-related mortality rate associated with intraperitoneal transfusion, especially for severely affected fetuses at less than 25 weeks' gestation, limited the need for the application of amniotic fluid analysis to the late second and early third trimesters of pregnancy.

During the last 5 years the development of safer techniques for fetal blood transfusions, either with the use of fetoscopy for direct intravascular access⁷ or by the intraperitoneal route with ultrasound guidance,^{8,9} and the improved prognosis for the very premature infant as a result of advances in neonatal intensive care, have encouraged the undertaking of obstetric interventions

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much earlier in pregnancy. In some centers, the Liley graph has been extrapolated backward and intrauterine transfusions are begun as early as 20 weeks, on the basis of these ΔOD_{450} values.⁸⁻¹⁰

The aim of the present study was to correlate amniotic fluid ΔOD_{450} values with the fetal hemoglobin in rhesus-isoimmunized pregnancies at 18 to 25 weeks to examine the predictive value of the ΔOD_{450} as well as the validity of extrapolating the original Liley graph from the third trimester to the middle of the second trimester of pregnancy.

Patients and methods

The study included 59 rhesus-negative pregnant women at 18 to 25 weeks' gestation who were considered to be severely rhesus isoimmunized on the basis of their obstetric history. These women were referred from several centers in the United Kingdom and Europe for direct intravascular fetal blood transfusion by fetoscopy because of elevated maternal serum rhesus antibody levels or amniotic fluid ΔOD_{450} measurements.

Linear-array real-time ultrasound scanning was performed to estimate gestational age, exclude external and internal anatomic defects, and detect fetal hydrops (skin edema and serous effusions). Fetoscopy and pure fetal blood sampling from an umbilical cord vessel was carried out with an Olympus Selfoscope 1.7 mm in diameter introduced transabdominally into the amniotic cavity.¹¹ Pure fetal blood samples were obtained from all fetuses and, after assessment of the degree of fetal anemia, with a combined electronic hemocytometer and particle size analyzer (Coulter Channelyzer C-100 with mean corpuscular volume-hematocrit attachment), an intravascular fetal blood transfusion was given. The data in this study were derived from pre-transfusion fetal blood and amniotic fluid samples obtained at the first fetoscopy. Further fetal blood transfusions were given as needed (mean number, three; range, one to six). The survival rate achieved in this series was 85%.

Fetal blood samples (180 μ l) were collected into 20 μ l of isotonic edetic acid solution (0.5 mmol/L in 0.15 mol/L sodium chloride) and the hemoglobin concentrations in grams per deciliter were determined with the use of a Coulter S-Plus counter. Amniotic fluid samples (10 ml) were collected at the time of fetoscopy and transferred into darkened containers to protect the bilirubin from photodecomposition. Samples were centrifuged for 15 minutes at 500 g to remove vernix caseosa, amniocytes, and erythrocytes and analyzed within 24 hours of collection. A spectrophotometric scan of the amniotic fluid was made with a double-beam, continuous-recording spectrophotometer (Pye Unicam SP 800). Optical density readings were plotted automatically on semilogarithmic graph paper, with

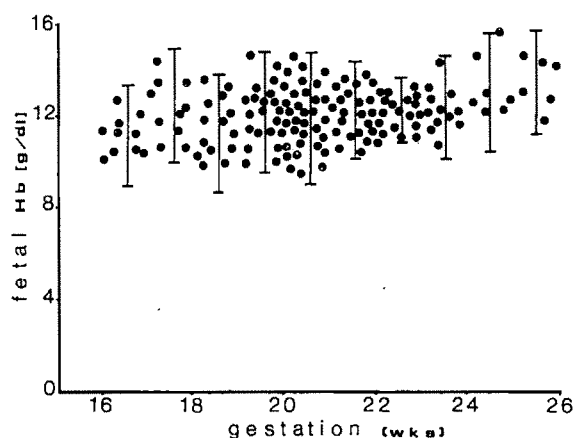


Fig. 1. Reference range (mean + 2SD) and distribution of individual values of fetal hemoglobin concentration from 153 pregnancies not complicated by fetal hemolysis.

wavelength as the linear abscissa (horizontal) and optical density deviation as the logarithmic ordinate (vertical). The bilirubin absorption band was measured manually as the vertical distance between the absorbance at 450 nm and the line connecting the curve between 550 and 365 nm.³ In 25 patients, another amniotic fluid sample, obtained by amniocentesis between 6 and 16 days before the fetoscopy, was also analyzed and the ΔOD_{450} determined.

Normal reference ranges for amniotic fluid ΔOD_{450} and fetal hemoglobin between 16 and 36 and between 16 and 25 weeks were established from 475 amniotic fluid and 153 fetal blood samples, respectively, obtained from pregnancies not complicated by fetal hemolysis. The mean and standard deviation for successive weeks of pregnancy and the correlation coefficient between each variable and fetal maturity in weeks were calculated. The probability of the correlation coefficient being significant was determined by Student's *t* test.

Results

The distribution of fetal hemoglobin and amniotic fluid ΔOD_{450} measurements from pregnancies not complicated by fetal hemolysis is shown in Fig. 1 and 2. Within the gestational range of 16 to 25 weeks, there was no significant change in either fetal hemoglobin (mean = 12.14, SD = 1.22) or liquor ΔOD_{450} (*n* = 246, mean = 0.103, SD = 0.045). For the log-transformed values of liquor ΔOD_{450} between 26 and 36 weeks' gestation, however, there was a highly significant negative linear correlation (*n* = 229, correlation coefficient *r* = -0.738, intercept = 2.776, slope = -0.127, *p* < 0.0001).

The amniotic fluid ΔOD_{450} values from the 59 rhesus-isoimmunized pregnancies are plotted against gestation (on the semilogarithmic graph) in Fig. 3 and divided

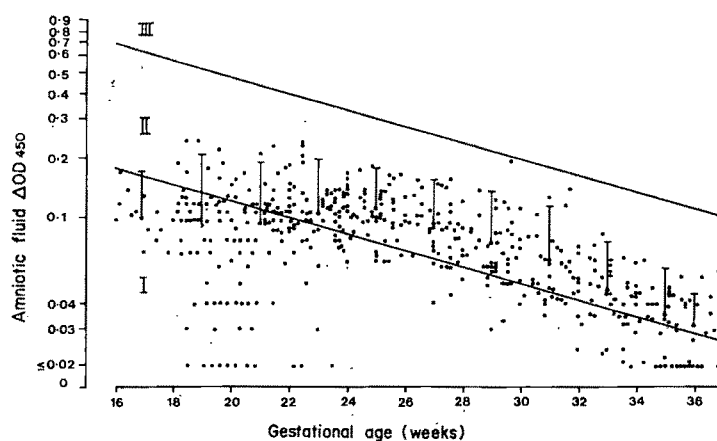


Fig. 2. Reference range (mean + 2SD) and distribution of 497 values of amniotic fluid ΔOD_{450} obtained from pregnancies not complicated by fetal hemolysis.

Table I. Accuracy of amniotic fluid ΔOD_{450} measurements in predicting severity of rhesus isoimmunization, as defined by fetal hemoglobin, in 59 pregnancies at 18 to 25 weeks' gestation

	Fetal hemoglobin concentration					
	<6 gm/dl		6-9.7 gm/dl		>9.7 gm/dl	
	n	%	n	%	n	%
Cutoff value of ΔOD_{450}						
>0.1	31*	100	15	100	13	100†
>0.2	28	90	13	87	7	54
>0.3	22	71	10	67	2	15
>0.4	13	42	6	40	2	15
>0.5	7	23‡	3	20	0	0§
Liley zone						
1	2	6	0	0	0	0
2A	3	10	4	27	8	62
2B	16	52	7	47	4	31
3	10	32	4	27	1	8

There is no ΔOD_{450} value (upper half) or extrapolated Liley zone (lower half) that clearly separates severely affected fetuses (hemoglobin <6 gm/dl) from nonanemic ones (hemoglobin >9.7 gm/dl). If the cutoff level is set at 0.1 nm in order to achieve detection of all 31 severely anemic fetuses(*), the false positive rate would be 100%(†). At ΔOD_{450} >0.5 nm the false positive rate is 0%(‡) but the detection rate is simultaneously reduced to 23%(§). Similarly, if obstetric intervention(s) is/are restricted to those pregnancies with amniotic fluid ΔOD_{450} values in extrapolated Liley zone 3 only 10(||) of the 31 severely anemic fetuses would be identified (32%).

into three prognostic zones by extrapolating the Liley lines backward to meet the vertical axis at 18 weeks' gestation; zone 2 is further subdivided into upper and lower subzones.⁴ The ΔOD_{450} values are correlated with the severity of the disease as defined by the fetal hemoglobin and the presence or absence of fetal hydrops at the time of sampling. Thus, if the hemoglobin fell within the normal range (<9.7 gm/dl) the disease was considered to be mild and the fetus therefore not in need of blood transfusion. If the hemoglobin was <6 gm/dl the disease was thought to be severe and the fetus at high risk of intrauterine death. Ultrasonographic evidence of hydrops was found in 14 of the 31 fetuses in this group. Moderate disease was defined by a fetal hemoglobin of 6 to 9.7 gm/dl. Although not at risk of imminent death, these fetuses would probably require an intrauterine blood transfusion within a few weeks.

The accuracy of predicting the severity of fetal anemia, and thus the need for obstetric intervention, by the Liley method and by specified cutoff levels of amniotic fluid ΔOD_{450} are shown in Table I. There is no ΔOD_{450} level that clearly separates severely from mildly affected pregnancies. If the cutoff level is set low enough to include 90% of the severely affected pregnancies, for example, at ΔOD_{450} >0.2 nm, the upper level of the normal ΔOD_{450} range (Fig. 2), it would also include 54% of the mildly affected ones. Alternatively, if the cutoff level is set high enough to exclude all mildly affected pregnancies (ΔOD_{450} >0.5), then the false negative rate would be 77%.

There is an overall negative linear correlation ($n = 59$, $r = -0.310$, $p < 0.05$) between fetal hemoglobin and ΔOD_{450} . Separate analyses of fetal hemoglobin and ΔOD_{450} in hydropic and nonhydropic fetuses reveal a

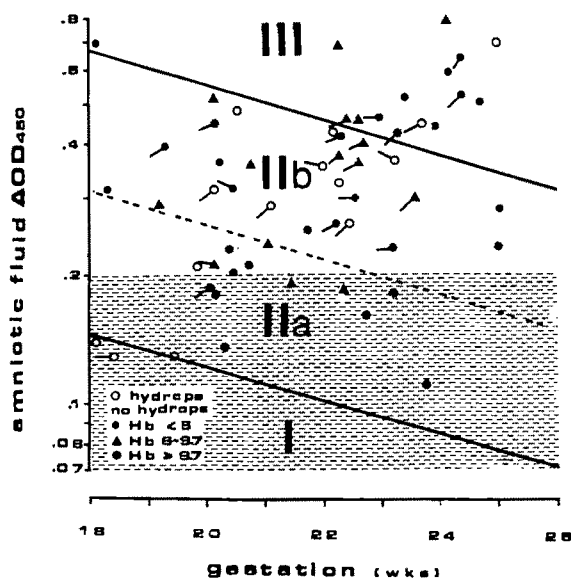


Fig. 3. Amniotic fluid ΔOD_{450} values and fetal hemoglobin concentration in 59 rhesus-isoimmunized pregnancies plotted on the extrapolated Liley graph. Open circles indicate hydropic fetuses. In 25 patients who had a prior ΔOD_{450} measurement, a short line denotes the slope of change.

highly significant positive correlation for the former ($n = 14$, $r = 0.750$, $p < 0.01$) and a negative one ($n = 45$, $r = -0.487$, $p < 0.001$) for the latter group of fetuses (Fig. 4). Thus the pregnancies with the most severely anemic hydropic fetuses had low values of liquor ΔOD_{450} . Ultrasonography, by identifying the hydropic fetuses, increases the sensitivity of elevated ΔOD_{450} measurements in detecting severely anemic fetuses from 32% to 71% (14 hydropic fetuses irrespective of ΔOD_{450} values plus eight nonhydropic ones with ΔOD_{450} values in Liley zone 3) without increasing the false positive rate.

The trends in ΔOD_{450} for the 25 patients who had had an amniocentesis 6 to 16 days before fetoscopy are illustrated in Fig. 3. In the 19 cases with measurements in the extrapolated Liley zones 1 and 2, the severity of fetal anemia could not have been reliably predicted by the trend of ΔOD_{450} values. Thus in four of the 11 (36%) severely affected pregnancies the trend in optical density deviation was downward. Moreover, of the 14 cases of ΔOD_{450} values that remained the same or increased, seven had severe disease, four moderately severe disease, and three mild disease.

Comment

In the present study the fetal hemoglobin, measured in samples obtained fetoscopically, was used as the basis for evaluating the severity of rhesus isoimmunization. The fetal hemoglobin rather than the outcome of pregnancy was chosen because: (1) fetal hemolysis and consequent anemia constitute the underlying pathophysiology of the disease¹²; (2) in the natural history of the

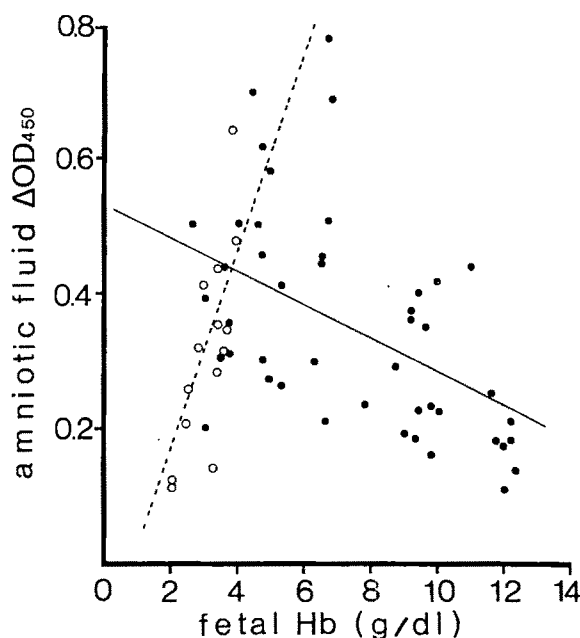


Fig. 4. Correlation of amniotic fluid ΔOD_{450} and fetal hemoglobin in 59 rhesus-isoimmunized pregnancies between 18 and 25 weeks' gestation. There is a highly significant positive correlation for the hydropic fetuses (○) and a negative one for the nonhydropic fetuses (●).

condition cord blood hemoglobin at the time of delivery has been found to be an accurate prognostic indicator for postnatal survival¹⁵; and (3) the currently achievable survival rate for infants of such pregnancies is greatly influenced by the antenatal treatment selected and the level of neonatal facilities and expertise available.

Since the normal range of fetal hemoglobin (Fig. 1) and ΔOD_{450} (Fig. 2) did not change within the gestational range of 16 to 25 weeks, the data derived from the rhesus-isoimmunized pregnancies could be analyzed independently of gestational age. The significant negative correlation between the two parameters (Fig. 4) supports the earlier work of Bevis,¹ Walker,² and Liley³ that the bilirubin concentration of the amniotic fluid reflects the degree of fetal hemolysis and is thus an indirect measure of fetal anemia. In the third trimester of pregnancy, the accuracy of predicting the severity of the disease by the Liley zone 3 method was found to be 94.5%.¹⁴ However, our data cast serious doubt on the validity of extrapolating the same criteria to the second trimester. Of the 31 most severely anemic fetuses (hemoglobin < 6 gm/dl), which included 14 with hydrops, only 10 (32%) would have received intrauterine blood transfusions at the time of sampling had the "extrapolated" Liley criteria been used (Table I). Although the predictive accuracy of the test is increased by performing serial amniocenteses and determining the trend in ΔOD_{450} ,⁵ the false positive and negative rates (Fig. 3) would remain unacceptably high. In the third trimester of pregnancy, the upper limit of ΔOD_{450}

values in pregnancies not complicated by fetal hemolysis decreases with gestation (Fig. 2) and lies in the upper subdivision of Liley's zone 2. For the second trimester, however, this limit is horizontal and coincides with the action line for fetal transfusions advocated by other investigators (for instance, 0.2 nm by Gordon et al.¹⁵). Had we adopted the criteria of these investigators, seven of the 13 (54%) mildly affected fetuses might have received unnecessary and potentially hazardous transfusions, while three (10%) of the severely anemic fetuses would not have been transfused (Table I). The positive correlation between fetal hemoglobin and ΔOD_{450} in the pregnancies complicated by fetal hydrops (Fig. 4) could be explained by the dilutional effect of associated polyhydramnios. This problem has been clearly demonstrated by Queenan,⁶ who advocated the determination of the amniotic fluid volume, when polyhydramnios is suspected, to allow appropriate correction of the measured ΔOD_{450} . Similarly, Whitfield¹⁶ found that, although the estimated amniotic fluid volume is within the normal range for gestation in most rhesus-isoimmunized pregnancies, rapidly developing polyhydramnios, which may even precede the development of hydrops, is usually associated with severely or fatally affected fetuses. Ultrasonography allows the diagnosis of fetal hydrops, thereby identifying a group of severely anemic fetuses in urgent need of blood transfusion. In the absence of hydrops, however, neither ΔOD_{450} (Table I) nor ultrasonographic measurements of intrauterine volume, placental thickness, umbilical vein diameter, fetal abdominal circumference, or intraperitoneal volume can reliably distinguish mild from severe fetal disease (unpublished results).

In the management of rhesus-isoimmunized pregnancies, it has traditionally been recommended that the first amniocentesis be performed 10 weeks before the time of the earliest previous fetal or neonatal death, fetal transfusion, or birth of a severely affected baby.¹⁶ In the absence of such a history, amniocentesis is undertaken when a "critical level" of maternal rhesus antibody is reached. A value of 4 IU/ml has recently been recommended.¹⁷ In the third trimester, amniocentesis and interpretation of ΔOD_{450} values by the Liley method have proved to be reliable. Fetal blood sampling may be associated with a marginally greater risk

of fetal mortality and maternal morbidity than amniocentesis.¹⁷ However, our present findings suggest that fetal hemoglobin determination on samples obtained with this technique is the only accurate method to assess the severity of fetal anemia in the second trimester of pregnancy.

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Endocrine aspects of human uterine sarcoma: A preliminary study

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The biologic effect of estrogen and progesterone in human uterine sarcoma is poorly understood in comparison to that of endometrial adenocarcinoma. In an attempt to elucidate the endocrine status of these tumors, we have investigated the ability of these tumors to synthesize estrogen by measuring the aromatase activity and studied the effect of aromatase inhibitors on the activity. In addition, the effect of estrogen and progesterone on aromatase activity and the growth pattern of these tumors were studied in cell culture and athymic mice systems. Aromatase activities in eight uterine sarcomas ranged from 0.7 to 37 fmol/hr $\times$ mg protein, which were within the range or higher than the activity found in normal proliferative endometrium (0.5 to 3 fmol/hr $\times$ mg of protein, $\bar{x}$ = 1.6, n = 10). These results indicate that uterine sarcomas are capable of producing estrogen. However, the enzyme activity showed no correlation with the morphology of tumors or the age of patients. Results from the kinetic studies of aromatase activity in one of the uterine sarcomas indicated that 19-nortestosterone, testolactone, and aminoglutethimide (the most effective one) inhibited aromatase activity. In addition, induction of aromatase activity in two uterine sarcomas was investigated in cell cultures. Progesterone caused an eightfold increase in activity in a sarcoma that was estrogen and progesterone receptor positive but had no effect in a tumor that was estrogen and progesterone receptor negative. The growth rate of two estrogen/progesterone receptor-negative uterine sarcomas was studied in cell culture and in athymic mice. Progestin, but not estrogen, reduced the growth rate in both systems; 30 nmol/L of estrogen had no effect on the growth rate. In summary, we have found that human uterine sarcoma is able to synthesize estrogen. Progesterone is able to induce the aromatase activity in estrogen/progesterone receptor-positive tumors, and progesterone also suppresses the tumor growth rate in estrogen/progesterone receptor-negative tumors. These results suggest that a select group of uterine sarcomas is sensitive to steroid hormone and that progesterone may be potentially beneficial for therapeutic treatment of select uterine sarcomas. (AM J OBSTET GYNECOL 1986;155:95-101.)

Key words: Uterine sarcoma, estrogen, progestin

Human uterine sarcomas represent rare gynecologic malignancies of poor prognosis. Their etiology is unknown, and little is known of their natural history other than their aggressiveness and propensity for apparent blood-born metastasis. The biologic parameters of these tumors remain poorly understood. The estrogen and progesterone receptor content has been reported in a few cases.¹⁻³ Most tumors have been shown to be hormone receptor negative. The negative receptor status would suggest that these tumors are hormone in-

sensitive, but the diversity of morphology seen in these tumors leads us to believe generalizations would be unsafe without further attempts to define the hormonal characteristics of these cancers.

The ability of these tumors to synthesize estrogens has been studied since we have found that various types of human endometrium are capable of synthesizing estrogen from Δ^4 -androgen.⁴⁻⁷ In normal endometrium a large variation of the aromatase activity was noted in two types of endometrial cells, epithelial glands and stromal cells, with the activity found predominantly in stromal cells. This finding led us to postulate that in sarcoma the malignant stromal cells may contain aromatase. In addition, we have conclusively demonstrated that aromatase in endometrial stromal cells is regulated by progesterone and that estrogen enhances this stimulation.⁷ Investigation of aromatase in uterine sarcomas may provide evidence to determine the hormone sensitivity of a given tumor.

Estrogen production *in situ* would increase the local

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Table I. Aromatase activity and estrogen and progesterone receptor content of mixed müllerian tumors

Specimen No.	Age (yr)	Morphology	Aromatase (fmol/hr × mg protein)	Cytosol receptors (fmol/mg protein)	
				Estrogen	Progesterone
1*	72	Homologous	6.3	14	830
2	68	Homologous	1.8	Negative	Negative
3	70	Homologous	1.0	Negative	Negative
4	19	Homologous	9.0	60	695
5	76	Heterologous	2.3	Negative	Negative
6*	64	Heterologous	5.0	—	—
7	74	Heterologous	0.7	—	—
8*	63	Heterologous	37	—	—

*Patient died of disease.

Table II. Kinetic constants of endometrial aromatase*

Types of endometrium	Michaelis-Menten constant (μmol/L)	Inhibition constant of aminoglutethimide (μmol/L)	Inhibition constant of 19-nortestosterone (μmol/L)	Inhibition constant of testolactone (μmol/L)
Normal stromal cells	0.02	0.5	—	
Decidua	0.024	0.5	0.5	
Uterine sarcoma, specimen 8	0.40	0.3	1.3	3.0

*Estimated from Figs. 1 and 2.

estrogen concentration, which can promote tumor growth in an estrogen-sensitive tumor. We have investigated the effectiveness of various aromatase inhibitors in altering the rate of estrogen production in a sarcoma with known high level of aromatase activity. These results may benefit in vivo management of estrogen sensitive tumors.

In addition, the effect of estrogen and progesterone on the growth rate in two specimens was studied in cell culture and in athymic mice systems in an effort to elucidate the direct effect of these two hormones on sarcoma.

Material and methods

Sarcoma specimens and isolation of endometrial cells. Specimens of uterine sarcoma were obtained from patients who were undergoing hysterectomy for medical treatment. Each specimen was thoroughly examined grossly as well as microscopically. A portion of the specimen was taken for aromatase activity and receptor assay. Viable tumor was also used for cell culture and transplantation in athymic mice when the size of the tumor was adequate for these studies. Histology, patient's age, and biochemical data are summarized in Table I.

Uterine sarcoma cells were isolated from a portion of specimens 1 and 5 (Table I) by digesting the tissue fragments in 0.1% collagenase solution as previously described.⁹ These cells were cultured in medium A

(medium RPMI-1640 [Gibco], 10% fetal calf serum [HyClone], 0.2 U/ml of insulin, 100 U/ml of penicillin, and 100 μg/ml of streptomycin) to study the induction of aromatase and the growth rate under the influence of estrogen and progesterone.

Assay of the aromatase activity and estrogen and progesterone receptor. Aromatase activity was measured in a cell-free system by incubating the tissue or cell homogenate with 100 nmol/L of tritiated testosterone (specific activity, 25 Ci/mmol, New England Nuclear) and 1 mmol/L of nicotinamide adenine dinucleotide phosphate in 0.05 mol/L of phosphate buffer (pH 7.4) for 3 hours at 37° C. The rate of aromatization has been shown to remain constant up to 4 hours under the assay condition.⁹

Various types of tritiated estrogens (estrone, and estradiol) converted from tritiated testosterone were purified and quantitatively isolated from each incubation by methods described previously.⁴ For the quantification of tritiated estrogens, carbon 14-labeled estrone and estradiol (New England Nuclear), which were added to these samples at the end of incubation, were used as internal standards.

The ability of various aromatase inhibitors to inhibit the aromatase activity was determined kinetically by means of the particulate fraction of specimen 8. Aminoglutethimide (Ciba-Geigy, Inc.), testolactone (Squibb) and 19-nortestosterone (Sigma Chemical Co.) were used in this study.

Determination of estrogen receptor and progesterone receptor. Sarcoma tissue fragments were homogenized (1 gm of tissue/10 ml) in Tris-hydrochloric acid buffer (0.01 mol/L, pH 7.4) containing 1.2 mmol/L of ethylenediaminetetraacetate and 0.5 mmol/L of dithioerythritol (TED). Cytosol was obtained from 100,000 × g supernatant of the tissue homogenate. Estrogen receptors in cytosol were measured by incubating the cytosol with tritiated estradiol (specific activity, 93 Ci/mmol, New England Nuclear) at concentrations ranging from 0.8 to 4 nmol/L, with and without a hundredfold excess of diethylstilbestrol. Incubations were carried out at room temperature, 22 to 25° C for 2 hours and 4° C for another hour. Dextran (0.005%)-coated charcoal (0.5%) suspension was used to separate the bound and free tritiated estradiol.

Progesterone receptors were measured in the same manner by incubating the cytosol with tritiated R-5020 (New England Nuclear) (0.8-4 nmol/L) with or without a hundredfold excess of cold R-5020 at 4° C for 4 hours in TED buffer containing 10% glycerol. Sodium molybdate, 10 mmol/L, was included in the incubation solution to stabilize both estrogen and progesterone receptor proteins. Receptor content and dissociation constant of estrogen and progesterone receptors were estimated from the Scatchard plot.

Growth pattern of endometrial sarcoma in athymic mice. Tumor fragments of specimens 3 and 5 were separately transplanted into athymic mice (Charles River Laboratories) with use of a modification of the method described by Satyaswaroop et al.¹⁰ Briefly, viable tissue fragments, ~100 mg, were minced in medium A and subcutaneously transplanted in the infrascapular region of castrated male nude mice with use of a 14-gauge syringe. The athymic mice were kept in cages and placed in a laminar hood to maintain an aseptic environment. Tumor size was estimated semi-weekly or weekly by measuring the mean diameter and weighing at the time when the mice were killed. The morphology and estrogen and progesterone receptor content were compared in specimen 5 in original tumor and in the tumors grown in the athymic mice. The effect of progesterone and estrogen on the growth of uterine sarcoma in athymic mice was carried out by implanting progesterone or estradiol pellets (Innovative Research, Bethesda, Maryland). The pellets release progesterone or estradiol at a rate that results in plasma levels of progesterone, ~10 ng/ml, or estradiol, 0.2 ng/ml, up to 60 days.

Results

Aromatase activity and estrogen and progesterone receptor content in uterine sarcomas. The age of patients, morphology, aromatase activity, and estrogen

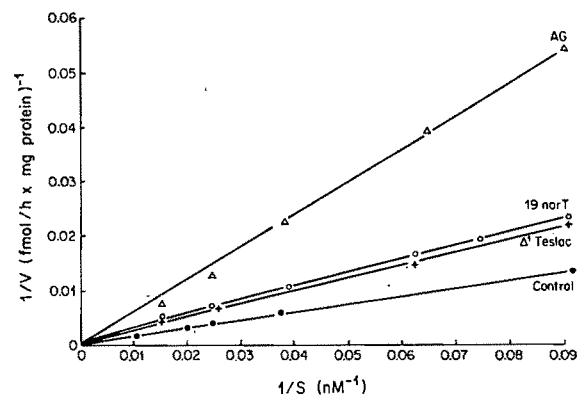


Fig. 1. Inhibition of aromatase activity by aminoglutethimide (AG) (2 μ mol/L), 19-nortestosterone (19-nor T, 1 μ mol/L), and testolactone (Δ^1 Testlac) (2 μ mol/L) in the particulate fraction of specimen 8. Lineweaver-Burk plot of aromatization of tritiated testosterone to estrogens (estrone and estradiol).

and progesterone receptor contents are shown in Table I. The aromatase activities in these specimens ranged from 0.7 to 37 fmol/hours × mg of protein. These values were either within the range or higher than the activity in normal proliferative endometrium (0.5 to 3 fmol/hr × mg of protein, $\bar{x}$ = 1.4, n = 10, unpublished observations). These results indicated that uterine sarcoma is able to synthesize estrogen. The production rate showed no clear correlation with the morphology of tumors or age of patients. Estrogen and progesterone receptor content, measured in five specimens, clearly indicate that the higher aromatase activity resided in the two specimens where receptor contents were positive. In specimen 5 we have further investigated the nuclear estrogen and progesterone receptor content, and found that it contained a small amount of estrogen receptor (130 fmol/mg of deoxyribonucleic acid) but nuclear progesterone receptor was negative. The transplanted tumor (specimen 5, second passage from athymic mice) was also estrogen/progesterone receptor negative.

Kinetic characteristic of aromatase of normal endometrium and uterine sarcoma and the effect of aromatase inhibitors on the rate of aromatization. Table II shows the apparent Michaelis-Menten constant of aromatase progesterone-stimulated normal endometrial stromal cells, decidua, and uterine sarcoma of specimen 8. The apparent Michaelis-Menten constant value in the uterine sarcoma obtained from the reciprocal plot of V vs. S, Fig. 1, was found to be approximately twentyfold higher than that of normal endometrium (Fig. 2) indicating that the characteristics of this enzyme are altered in sarcoma. Inhibition constants of aminoglutethimide, 19-nortestosterone and testolactone for normal endometrium and uterine sarcoma are also

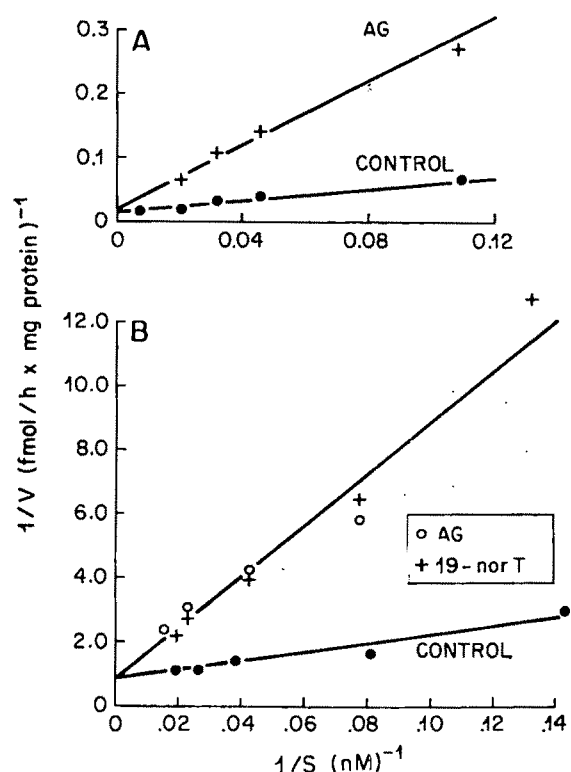


Fig. 2. Inhibition of aromatase activity by aminoglutethimide and 19-nortestosterone (2 $\mu\text{mol/L}$) in cultured endometrial stromal cells (A) and in first-trimester decidua (B). Lineweaver-Burk plot of aromatization of tritiated testosterone to estrogens (estrone and estradiol).

summarized in Table II. Both aminoglutethimide and 19-nortestosterone are equally effective in inhibiting aromatase activity in normal endometrium and uterine sarcoma, and testolactone is less effective than aminoglutethimide and 19-nortestosterone.

Effect of estrogen and progesterone on aromatase and cell growth in isolated uterine sarcoma cells in culture. The uterine sarcoma cells of specimens 1 and 5 (shown in Table I) were successfully isolated and cultured in nutrient medium. Progesterone was added to the culture medium and the aromatase activities were measured in these cells after 2 or 4 days of hormone treatment (Table III). Progesterone caused an eight-fold increase of aromatase activity in specimen 1 (estrogen/progesterone receptor positive) while no effect was noted in specimen 5 (estrogen/progesterone receptor negative).

The growth pattern of specimen 5 was examined in culture. Fig. 3 shows the growth rate of this sarcoma under three different culture conditions: control, medium supplemented with 30 nmol/L of estradiol, and 1 $\mu\text{mol/L}$ of medium supplemented with medroxyprogesterone acetate. The rate of growth in cells supplemented with estradiol was found to be similar to the

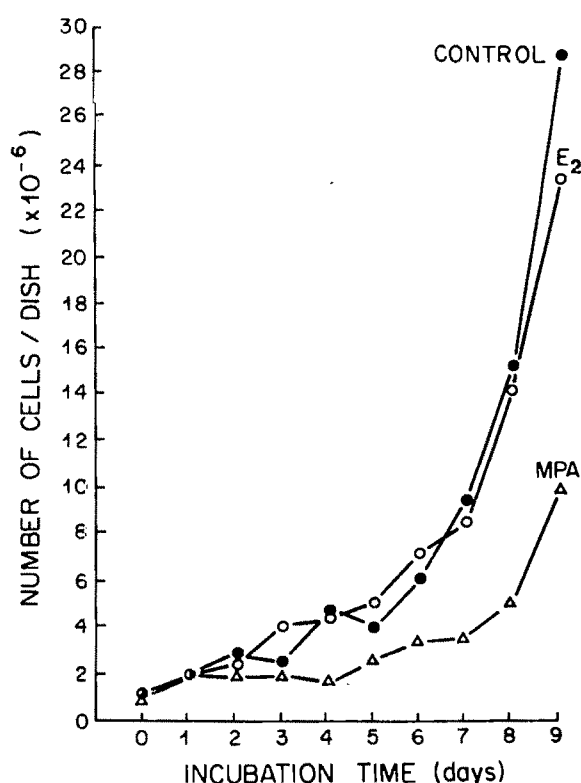


Fig. 3. Growth rate of uterine sarcoma in cell culture. Sarcoma cells were isolated from specimen 5 and cultured in medium A until confluent. These cells were then evenly distributed into 60 dishes (10 $\times$ 60 mm), steroid hormones were added to Petri dishes after cells have been attached to Petri dishes, 6 hours after plating, 20 dishes for control, estradiol (E_2 , 30 nmol/L) and medroxyprogesterone acetate (MPA, 1 $\mu\text{mol/L}$), respectively. Medium was renewed every day, and cells were counted each day by a hemocytometer. Data represent mean value of duplicate counting from two separate dishes.

control, indicating that the growth rate of this sarcoma was not affected by 30 nmol/L of estrogen. However, the growth rate in cells cultured with medroxyprogesterone acetate was reduced to less than 30% of control at the end of 9 days of culture. The mechanism by which medroxyprogesterone acetate suppresses the cell growth is unknown.

Morphology, growth pattern, and aromatase activity of uterine sarcoma transplanted into athymic mice. Specimens 3 and 5 were transplanted into athymic mice. Heterotransplantation was successful in all grafts from the original tumor. The morphology of the original and transplanted tumors of specimen 5 are shown in Figs. 4 and 5. The original tumor was composed predominantly of poorly differentiated sarcoma consistent with endometrial stromal sarcoma (Fig. 4). Foci of malignant cartilage were present. A minor (less than 1%) component consisted of adenosquamous carcinoma. The transplanted tumor showed similar histol-

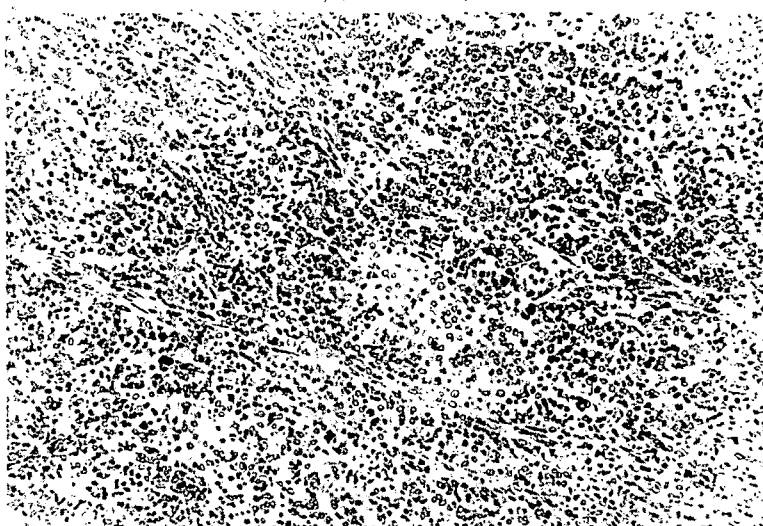


Fig. 4. Morphology of the original tumor of specimen 5. Malignant tumor composed of sheets of poorly differentiated endometrial stromal type cells. Lighter staining area shows cartilaginous differentiations. (Original magnification $\times 100$.)

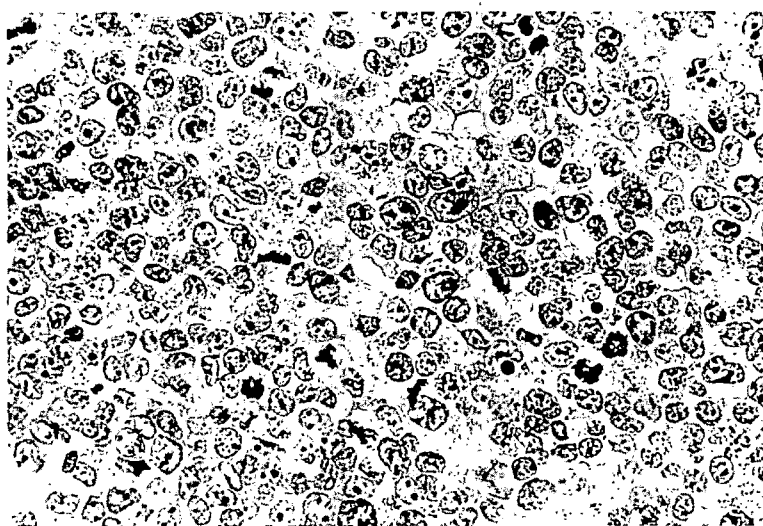


Fig. 5. Morphology of transplanted sarcoma, specimen 5, after second passage. Sheets of malignant endometrial stromal cells similar to those present in original tumor, Fig. 4. (Original magnification $\times 250$.)

Table III. Effect of progesterone on aromatase activity in culture*

Specimen No.	Compound added to culture medium	Incubation time (day)	Aromatase activity (fmol/hr $\times$ mg of protein)
1	Control	—	2.0
	Progesterone (1.8 μ mol/L)	4	16.0
5	Control	—	6.3
	Progesterone (1.8 μ mol/L)	2	7.0

*Stromal cells were isolated from specimens 1 and 5 and cultured in medium A till confluent. Progesterone was added to the cultured medium, and cells were continuously cultured. Aromatase activity was determined at the end of 2 and 4 days. Data represent mean value of two determinations from two separate dishes.

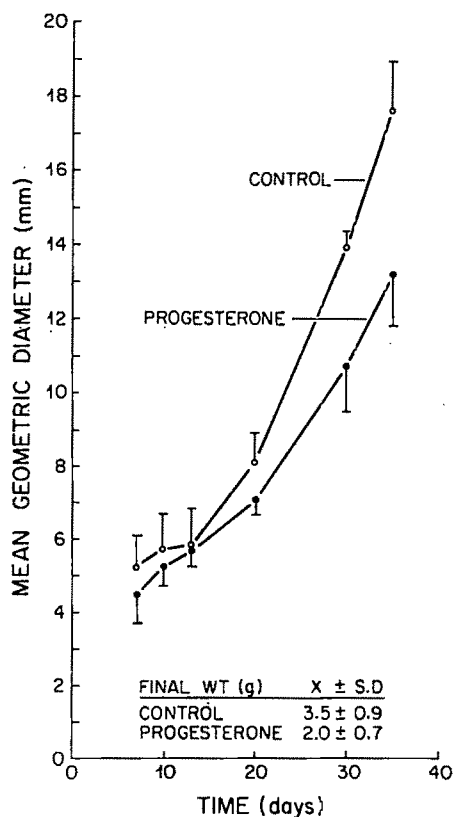


Fig. 6. Effect of progesterone on the growth of human uterine sarcoma. About 100 mg of tissue, second passage of specimen 3, were subcutaneously transplanted in the infrascapular region of six castrated mice. Three of the animals served as controls while the other three received subcutaneous implants of progesterone pellet. The tumor volume was determined semiweekly with venier clippers.

ogy, being composed of endometrial stromal sarcoma with foci suggesting cartilaginous differentiation (Fig. 5). An epithelial element was not identified. The failure to find epithelial elements may reflect greater propensity for the sarcomatous component to proliferate in the transplants than the epithelial component.

The growth pattern under the influence of progesterone in tumors 3 and 5 was investigated in castrated mice. The growth rate is considerably faster than that of endometrial adenocarcinoma.¹⁰ Fig. 6 shows the growth rate of specimen 3. The weight of the tumor after 35 days of transplantation was 3.5 ± 0.9 gm (mean $\pm$ SD, $n = 3$) in control mice. The growth rate of the tumor was found to be considerably slower in nude mice treated with progesterone. The weight of tumor in progesterone-implanted mice at the end of 35 days was 2.0 ± 0.7 gm ($n = 3$). Similar results were obtained for specimen 5 (data not shown). The final weights of tumors after 3 weeks of period were 3.4 and 1.7 gm in control and progesterone-treated mice, respectively. The growth patterns of specimens 3 and 5

in mice implanted with estradiol were similar to that of controls (data not shown). These results are in agreement with the findings in cell culture (Fig. 3). In addition, three weeks' treatment with progesterone or estradiol had no effect on aromatase activity in these tumors.

Comment

Our present studies, though limited to a small number of tumors, demonstrated that uterine sarcomas contain aromatase activity, and that four of eight were found to have higher activity than found in normal endometrium. The presence of this enzyme in uterine sarcoma implies that these tumors may be sensitive to progestin during malignant transformation, since aromatase activity in normal endometrium is regulated by progestin. As indicated in the cell culture system, progesterone induced aromatase activity in specimen 1, consistent with the receptor status. These results suggest that induction of aromatase is mediated through the interaction of receptor complex with genome. It is interesting to note that three tumor specimens with relatively high aromatase activity were obtained from patients who subsequently died of disease within 1½ years after operation.

Kinetic studies of aromatase in uterine sarcoma indicate that the conventional aromatase inhibitors effectively inhibit estrogen production in uterine sarcoma. This result suggests that a compound such as aminoglutethimide, which has been used for the treatment of breast tumor,¹¹⁻¹⁵ may be useful for regression of uterine cancer. The tumor growth may not be affected by estrogen in estrogen receptor-negative sarcoma as indicated in Fig. 3. However, in the case of an estrogen receptor-positive sarcoma or a mixed müllerian carcinosarcoma, aromatase activity of the sarcomatous component may provide locally sufficient amounts of estradiol to promote the tumor growth, especially if the adenocarcinoma component is estrogen receptor positive.¹⁶

Progestin therapy is known to be of benefit in some patients with endometrial adenocarcinoma, that is, in general, in well-differentiated estrogen/progesterone receptor-positive tumors in which the tumor regression is known to be mediated through the mechanism of progesterone receptors. In specimens 3 and 5, both of which were progesterone receptor negative, progesterone decreased the growth rate of tumor in both cell culture and athymic mice. These two sarcomas may represent a class of tumor in which the growth rate is regulated by progesterone but is independent of progesterone receptor in cytosol. It has been shown that the cell membrane of *Xenopus* oocytes contains progesterone receptor exclusion from the cytosolic receptor.¹⁷ Uterine sarcoma, although a very different tissue

from *Xenopus* oocytes, may also contain progesterone receptors on cell membrane to exert a destructive effect on these tumors. Although the mechanism of the tumor regression are unknown at the present time, our results suggest that select patients with mixed müllerian sarcomas may respond to progestational therapy regardless of the cytosolic estrogen/progesterone receptor status. The cellular biologic change caused by progestin that led to the reduction of tumor growth needs to be further studied to assess the multiple potential actions of progestin in gynecologic cancer.

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Disaturated phosphatidylcholine as a fetal lung maturity test: Lack of interference by seminal ejaculate as a contaminant in vaginally pooled amniotic fluid samples

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Seminal ejaculates obtained from four men were added to amniotic fluid pools. Lecithin/sphingomyelin ratios in amniotic fluid were significantly lowered in the presence of small amounts of contaminating semen. In contrast, the determination of disaturated phosphatidylcholine was not affected by the presence of this contaminant. (*AM J OBSTET GYNECOL* 1986;155:101-2.)

Key words: Semen, disaturated phosphatidylcholine, lung

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With contamination of amniotic fluid by blood or meconium, the quantitation of disaturated phosphatidylcholine, the surface-active species in total phosphatidylcholine, has proved to be a better predictor of fetal lung maturity than has the lecithin/sphingomyelin (L/S) ratio.¹ In instances of premature rupture of the membranes, a vaginal pool is often the only available

Table I. Effect of semen contamination on amniotic fluid L/S ratio and disaturated phosphatidylcholine determinations

Semen contamination (%, v/v of amniotic fluid)	L/S ratio of amniotic fluid pool		Disaturated phosphatidylcholine quantity of amniotic pool ($\mu\text{g}/100\text{ ml}$)		
	No. 1	No. 2	No. 1	No. 2	No. 3
0	$6.22 \pm 0.16^*$	3.04 ± 0.25	2153 ± 77	652 ± 33	$366 \pm 16^*$
2	3.69 ± 0.38	2.46 ± 0.64	1838 ± 219	618 ± 35	341 ± 3
5	2.64 ± 0.35	1.53 ± 0.27	2155 ± 208	611 ± 33	358 ± 72
10	1.90 ± 0.11	1.24 ± 0.20	2089 ± 160	589 ± 36	356 ± 43

*Data expressed as mean $\pm$ SEM for two to five determinations.

source of amniotic fluid. Frequently this pooled fluid is contaminated with blood or meconium; therefore, the quantitation of disaturated phosphatidylcholine is the test of choice for predicting fetal lung maturity. There is no information available, however, on the contamination of vaginal pools of amniotic fluid by seminal ejaculate and the subsequent impact on the quantitation of disaturated phosphatidylcholine.

Seminal ejaculate is rich in various phospholipids that interfere with the determination of L/S ratios. Romem et al.² recently studied human seminal ejaculate and reported this fluid to be rich in sphingomyelin. Thus, even at a 1/50 dilution, seminal ejaculate significantly lowered the L/S ratio of amniotic fluid. On the other hand, seminal ejaculate does not contain phosphatidylglycerol. Therefore, these authors suggested that in cases where recent coitus occurs before rupture of the membranes, the presence of phosphatidylglycerol is still indicative of fetal lung maturity while the results of the L/S ratio should be doubted. However, phosphatidylglycerol appears late in gestation. While the appearance of phosphatidylglycerol virtually guarantees the absence of respiratory distress syndrome, phosphatidylglycerol may be absent in more than 50% of the amniotic fluid samples with mature L/S ratios (>2.0 and <4.1).³ Therefore we investigated whether the measurement of disaturated phosphatidylcholine in amniotic fluid would be influenced by semen.

Seminal ejaculates were obtained from four men. Each sample was used individually and was added in varying quantities to pools of amniotic fluid. Two pools of amniotic fluid were used for L/S ratio determinations, while disaturated phosphatidylcholine was quan-

titated in three amniotic fluid pools according to our previously published methods.^{1,3} Table I shows the effect of seminal ejaculate on L/S ratio and disaturated phosphatidylcholine determinations in amniotic fluid. When amniotic fluid was contaminated with 10% seminal ejaculate, the mean L/S ratios in the two amniotic fluid pools were lowered from 6.22 and 3.04 to 1.90 and 1.24, respectively. This is in agreement with the finding of Romem et al.² In contrast, similar amounts of semen added to amniotic fluid did not affect the determination of disaturated phosphatidylcholine. When disaturated phosphatidylcholine determinations were carried out on undiluted individual semen samples, two of the four samples did not have any measurable amount of disaturated phosphatidylcholine, while the other two had minimal amounts (140 and 225 $\mu\text{g}/100\text{ ml}$, respectively). We conclude that disaturated phosphatidylcholine is not present in significant quantities in seminal ejaculates and that disaturated phosphatidylcholine determination is a useful fetal lung maturity test for vaginally pooled amniotic fluid even if the samples are suspected of being contaminated with seminal ejaculate.

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Peripartum cardiomyopathy: Echocardiographic features in five cases

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Five cases of peripartum cardiomyopathy are presented. All patients were aged <35 years, and four were multiparous. Two cases followed twin deliveries. Pulmonary embolism was diagnosed in four patients. Electrocardiograms showed left ventricular hypertrophy or left bundle branch block. On echocardiography, left ventricular minor axis dimensions were increased (diastolic, 67 ± 7 mm; systolic, 59 ± 7 mm) and mean fractional shortening was reduced ($13\% \pm 5\%$). All patients had hypokinesis or akinesis of the left ventricular segments and two had right ventricular dilatation. Gallium scanning performed in three patients was negative. Viral serologic testing was negative in all cases. All patients died within 5 years, three within 5 months. Survival duration was closely correlated with left ventricular fractional shortening. Autopsy in three patients confirmed the diagnosis of a dilated cardiomyopathy. (AM J OBSTET GYNECOL 1986; 155:103-6.)

Key words: Peripartum cardiomyopathy, echocardiography, gallium myocardial scintigraphy

The development of cardiac failure in the last trimester of pregnancy or the puerperium, in the absence of any recognizable etiology, is fortunately rare. Most series of peripartum cardiomyopathy have come from the southern regions of the United States,¹ South Africa,² and Jamaica.³ A recent review⁴ reported that about 90% of all reported cases were in black women, usually over 30 years of age and multiparous, with a high incidence of twin pregnancy. Pulmonary embolism was a common complication, and 5-year mortality ranged from 20% to 60%. The prognosis was inversely related to the degree of cardiomegaly on chest radiography at 6 months.

The etiology of peripartum cardiomyopathy is unknown, although a number of theories have been proposed,⁴ for instance, nutritional deficiency, pre-eclampsia and hypertension, genetic predisposition, autoimmune response, myocarditis, and drug-induced myocardial injury. The pathologic findings have included chamber dilatation and nonspecific histologic signs of fiber separation and myocyte necrosis and loss without significant cellular infiltrate.⁵ The management has included digoxin and diuretics, afterload reduction,⁶ anticoagulation,⁷ prolonged bed rest,⁸ and cardiac transplantation.⁹

We describe the clinical, electrocardiographic, and echocardiographic characteristics of five patients with peripartum cardiomyopathy. The echocardiographic

features of this disorder and their close correlation with the outcome have not been previously described.

Subjects and methods

Since 1978, our cardiology department has provided an echocardiographic service for a population of about one million in Queensland, the northeastern state of Australia. We also service the Royal Women's Hospital, the largest maternity hospital in Queensland. The computerized record system and clinical notes of all patients undergoing echocardiography during this period were searched for women who met the following criteria for peripartum cardiomyopathy⁴: (1) Cardiac failure developing during the last trimester of pregnancy or the first 20 weeks post partum, (2) absence of demonstrable heart disease before pregnancy, and (3) absence of a determinable cause of cardiac failure.

In determining left ventricular dilatation, echocardiography is more accurate and sensitive than the finding of cardiomegaly on chest radiographs (as used in previous series). Echocardiography may also be used to assess systolic function to exclude valvular causes of cardiac failure. Left ventricular minor axis dimensions were determined by standard echocardiographic techniques. Fractional shortening [(diastolic dimension - systolic dimension) $\times$ 100/diastolic dimension] has been shown to correlate with left ventricular ejection fraction.¹⁰ Segmental left ventricular function was assessed with the use of nine echocardiographic regions (apex; proximal, and middle portions of the septum, anterior, lateral, and inferior regions). Each region was graded as normal = 0, hypokinesis = 1, akinesis = 2, or dyskinesis = 3, with the sum of these grades expressed as the wall motion index.

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Table I. Demographic and clinical data

Case No.	Age (yr)	Race and birthplace	Parity	Twins	Onset of symptoms	Pulmonary embolism
1	35	Melanesian, Solomon Islands	3	+	At delivery	+
2	26	Caucasian, New Guinea	4	+	2 mo post partum	+
3	22	Caucasian, Australia	1	—	35 wk	+
4	27	Caucasian, Australia	2	—	4 mo post partum	+
5	34	Caucasian, Australia	2	—	2 mo post partum	—

Table II. Laboratory and survival data

Case No.	ECG	Gallium scan	Ds/Dd	Fractional shortening (%)	Wall motion index	Right ventricle	Survival (post partum)	Mode of death
1	LVH	Neg.	55/60	8	18	Dilated	3 mo	Cardiac failure
2	LVH	Neg.	60/68	12	15	Dilated	5 mo	Cardiac failure
3	LVH	Neg.	70/78	10	16	Normal	2 mo	Cardiac failure
4	LVH		57/65	12		—	15 mo	Sudden death
5	LBBB		52/66	21		—	5 yr	Ventricular fibrillation

ECG = Electrocardiogram; LVH = left ventricular hypertrophy; LBBB = left bundle branch block; Ds = minor axis left ventricular systolic diameter; Dd = minor axis left ventricular diastolic diameter; FS = fractional shortening (Dd/Ds)/Dd; Neg. = negative.

Myocardial scintigraphy with gallium 67 citrate was performed in three patients in an attempt to identify potential candidates for immunosuppressive therapy.¹¹

Simple linear regression was used for statistical evaluation.

Results

Of the 13,500 records searched, only five cases met the criteria for peripartum cardiomyopathy, and their demographic and obstetric details, clinical features, investigations, and course are shown in Tables I and II. Fig. 1 shows the correlation between echocardiographic fractional shortening and survival duration. Autopsies were performed in three patients. Three other women of reproductive age had the clinical and echocardiographic characteristics of dilated cardiomyopathy but had not recently been pregnant.

Case reports

Case 1. A 35-year-old Melanesian woman, born in the Solomon Islands, developed pleuritic chest pain and dyspnea after the vaginal delivery of twins. The pregnancy had been complicated by recurrent urinary tract infections. She had been normotensive but developed mild left ventricular failure at the time of delivery. She had not received betamethasone or tocolytic agents. She responded to intravenous furosemide and remained otherwise well for the first 2 weeks post partum. She did not use tobacco or alcohol and had no other history of chest pain. Examination revealed hypotension, a gallop rhythm, and biventricular failure. Echocardiography demonstrated thrombus within the inferior vena cava and right atrium as well as biventricular dilatation. A diagnosis of dilated cardiomyop-

athy and secondary pulmonary embolism were made and she was treated with digoxin, furosemide, dopamine, and anticoagulants. Endomyocardial biopsy showed mild interfibrillary edema. Viral serologic testing and tissue autoantibodies were negative. Her condition deteriorated with increasing left ventricular failure and cardiogenic shock, accompanied by some non-sustained episodes of ventricular tachycardia. She died in the hospital of cardiac failure 3 months post partum. Autopsy showed a dilated cardiomyopathy with no cellular infiltrate, minimal fibrosis, and normal coronary arteries.

Case 2. A 26-year-old Caucasian woman was admitted 3 months post partum with a history of weakness and nausea for 1 month and 2 days of severe pleuritic chest pain and hemoptysis. She had no previous history of heart disease and did not drink alcohol. She had been born in New Guinea but had lived for several years in Central Queensland. Twin girls had been delivered by cesarean section and the pregnancy had been otherwise uncomplicated. Examination revealed hypotension, a gallop rhythm, right pleural effusion, and hepatomegaly. She was treated with digoxin, furosemide, and dopamine. Ventilation-perfusion lung scanning confirmed pulmonary infarction and she underwent anticoagulation. Her condition deteriorated and she developed cardiogenic shock and renal failure. She died 5 months post partum. Autopsy showed normal coronary arteries, a severe dilated cardiomyopathy, and old pulmonary infarcts. Histologic analysis showed myocyte hypertrophy and a low-grade lymphocytic infiltrate.

Case 3. A 22-year-old Caucasian primigravid woman was transferred for cesarean section following the onset of severe left ventricular failure at 35 weeks' gestation.

There was no previous history of heart disease or shortness of breath. Her pregnancy was uncomplicated except for a brief "flu-like illness" at 24 weeks' gestation. Viral serologic testing was negative. Examination revealed a gallop rhythm and signs of left ventricular failure. Gated heart pool scan showed a left ventricular ejection fraction of 19%. She was discharged after 1 month on a regimen of digoxin, furosemide, and prazosin but was readmitted a week later with deep venous thrombosis and pulmonary embolism. She underwent anticoagulation. Captopril was commenced because of worsening cardiac failure but she died of cardiac failure 10 weeks post partum.

Case 4. A 27-year-old Caucasian woman, gravida 2, para 2, was referred for assessment of worsening cardiac failure. Her second pregnancy had been complicated by mild preeclampsia but was otherwise uneventful. She had no past history of heart disease or dyspnea. She developed left ventricular failure and peripheral edema 4 months post partum and was treated with digoxin and diuretics and empirically with oral prednisone for 6 weeks, although there was no clinical or serologic evidence of myocarditis. Nine months post partum she was admitted to the hospital with pulmonary embolism, which was confirmed by ventilation-perfusion lung scanning, and underwent anticoagulation. On examination she had a gallop rhythm, cardiomegaly, and biventricular failure. Viral serologic testing was negative. She improved with increased diuretics and was discharged. She was readmitted 2 months later with left ventricular failure and died of a cardiac arrest while in the hospital. Autopsy showed a dilated cardiomyopathy with separation of muscle bundles, no cellular infiltrate, and normal coronary arteries.

Case 5. A 34-year-old Caucasian women, gravida 2, para 2, developed severe left ventricular failure 7 weeks after a cesarean section. There was no previous history of heart disease and the pregnancy was otherwise uncomplicated. On examination she had a tachycardia, gallop rhythm, cardiomegaly, and lung crepitations. Cardiac enzymes, viral serologic testing, and tissue antibodies were negative. She was treated with digoxin, diuretics, and 6 weeks of bed rest, which resulted in symptomatic improvement, but there was no decrease in left ventricular diameter. She was discharged and remained on a regimen of digoxin, 0.5 mg daily, in mild cardiac failure, for 5 years but was then readmitted with left ventricular failure and died 3 days later of ventricular fibrillation.

Comment

Peripartum cardiomyopathy is rather rare in our community. In this series, compiled during a 7-year period and including referrals from the largest maternity hospital in Queensland, only five cases were identified among 13,500 echocardiograms. However, it accounted for a large proportion of the causes of dilated cardiomyopathy in women of reproductive age (five of eight). The mean age of the patients was 27 years: four

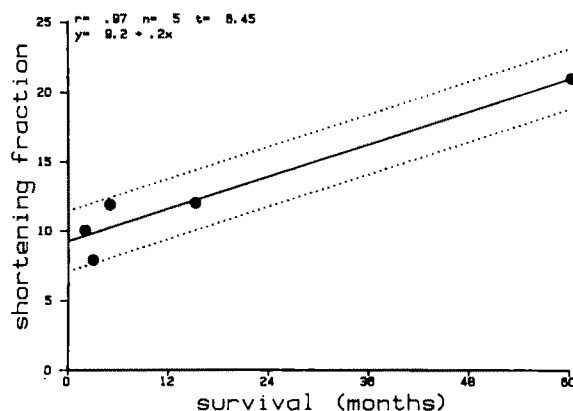


Fig. 1 Scatterplot and linear regression slope for left ventricular function (fractional shortening) versus survival duration ($p < 0.01$).

were Caucasian and one, Melanesian. As in previous series, the majority were multiparous (mean parity 2.4) and there was a high rate of twin pregnancy (two of five). One patient had a previous history of preeclampsia. Most of the patients developed cardiac failure in the postpartum period, although there was no relationship between the subsequent course and the timing of presentation. As in previous series, pulmonary embolism was common (four of five) and all patients had abnormal electrocardiograms with voltage criteria for left ventricular hypertrophy (four of five) or left bundle branch block (one of five). The 5-year mortality was 100%, compared with 20% to 60% in previous series.⁴ The autopsy findings were nonspecific.

This is the first series of peripartum cardiomyopathy in which the echocardiographic features and gallium scanning have been described. The initial echocardiographic left ventricular fractional shortening was closely correlated with the duration of survival ($r = 0.97$, $p < 0.01$). Right ventricular dilatation was not predictive of the subsequent course. Patients who died within 5 months of onset died of cardiogenic shock whereas those dying later had fatal arrhythmias. This has implications for treatment. Patients with severe left ventricular dysfunction and hypotension should be assessed for cardiac transplantation. Those patients with moderately reduced left ventricular function, without hypotension, may benefit from treatment for ventricular arrhythmias.

All patients presenting with acute onset of cardiomyopathy should be assessed for myocarditis, by means of gallium scanning¹¹ and/or endomyocardial biopsy,¹² to identify candidates for immunosuppressive therapy. Mason et al.¹² showed that 10% to 25% of patients with acute dilated cardiomyopathy had evidence of myocarditis on endomyocardial biopsy. In our series, negative myocardial gallium scans were found in three patients early in the illness. In the two cases in which both

gallium scans and autopsies were performed, the "cold" scan was consistent with the absence of myocarditis on histologic examination.

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A stillbirth with aspirated squamous pearls and fetoplacental thromboembolism: A new finding and reminder of the importance of placental and fetal pathologic conditions

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Fetal aspiration of squamous epithelial pearls has probably never been reported. Because of deficient perinatal pathology examinations, the occurrence of fetoplacental thromboembolism is incompletely known. This case report of the two phenomena includes emphasis that placentas should never be frozen; they should be stored at standard refrigerator temperature. (*AM J OBSTET GYNECOL* 1986;155:106-7.)

Key words: Stillbirth, aspirated squamous pearls, fetoplacental thromboembolism

We have performed many autopsies but have never seen a case in which lungs have included numerous squamous epithelial pearls. Consultation with colleagues and a literature search have not revealed description of this finding. The present case was a stillbirth that had been referred from another medical institute.

Case report

The mother was a 29-year-old, childless woman, who had experienced a previous midtrimester spontaneous abortion. On July 24, 1985, when 32 weeks' pregnant,

she was admitted to the hospital because of premature labor. The fetal heart was then audible. A diagnosis of abruptio placentae was made. Two days later, an 1850 gm nondysmorphic, stillborn female infant was delivered. There was a large quantity of old blood clots in the amniotic fluid. The 250 gm trimmed placenta was normally formed. There were lateral and basal signs of recent hemorrhage but no hemosiderin or infarcts. Freeze artifacts obliterated the placental amniotic surface and thereby prevented interpretation of histologic features. Despite artifacts, clumps of squamous cells were identifiable about the umbilical cord (Fig. 1, a), and meconium-laden macrophages were seen within the subamniotic connective tissue. The placenta and fetus showed signs of fetoplacental thromboembolism. Laminated fibrin was present across large fetal-placental veins. There was fibrin in umbilical cord vessels; in the fetal kidneys, liver, and spleen, hemorrhagic infarcts were adjacent to vascular fibrin thrombi.

In addition to numerous pearls of epithelial cells, the lungs included squamae and many uninucleate and bi-

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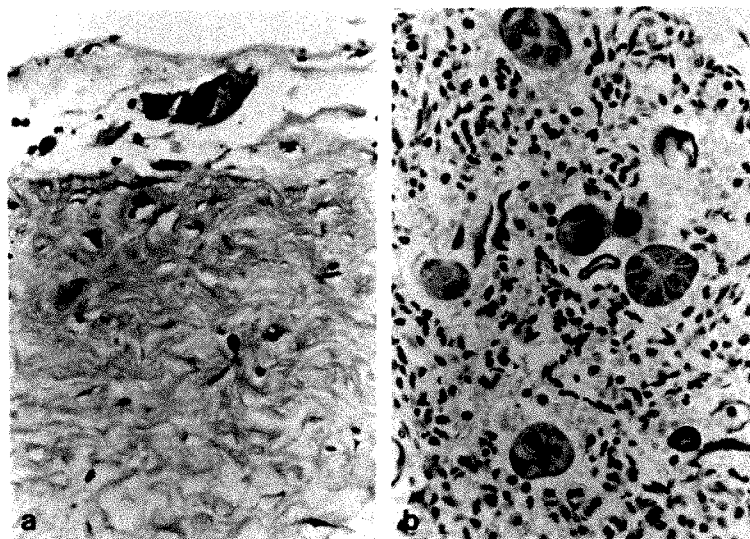


Fig. 1. *a*, Umbilical cord surface. Note the cluster of epithelial cells. (Hematoxylin and eosin. Original magnification $\times 200$.) *b*, Lung. Squamae and pearl-like clusters of epithelial cells are numerous present. (Hematoxylin and eosin. Original magnification $\times 200$.)

nucleate epithelial cells; we confirmed the nature of these pearls by labeling them with antikeratin antibody, conjugated with immunoperoxidase (Fig. 1, *b*). A comprehensive gross and light microscopic study was made, which included the central nervous system. There were no inflammatory or other noteworthy features, in the lungs or elsewhere.

Comment

Because the placenta had been frozen, it was difficult to establish the origin of the pulmonary, squamous epithelial pearls. Fortunately, several similar pearls were identifiable across the umbilical cord (Fig. 1, *a*). Placentas should be stored at 4° C, so that tissue artifacts can be minimized.

Light microscopic examination of the upper respiratory tract did not show unusual epithelial changes. Sloughing of fetal skin is not likely to have contributed to the findings in the lungs. Brown hair and lanugo were present across the head, and no lesions of aplasia cutis congenita were seen across the body. In that rare entity, skin is absent from the scalp or elsewhere.¹ In 113 of 400 reviewed cases, skin was absent at sites other

than the scalp, and there was an approximately 10% association with fetus papyraceus.¹

Autopsy examination of stillbirths often reveals pathologic processes; this is also true of associated placental examinations.² Perinatal pathologic findings must inevitably enhance knowledge of neonatal diseases. In the present case, for example, fetoplacental thromboembolism was apparent. Fibrin was located in several vessels of the placenta, umbilical cord, kidneys, liver, and spleen. Within the latter three organs, hemorrhagic infarcts were adjacent to the fibrin thrombi. A lack of similar lesions in the brain may have resulted in fibrinolysis.

Because of numerically deficient pathology studies, the importance of fetoplacental thromboembolism is insufficiently known.

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Immunologic factors contributing to the initiation of labor— Lymphocyte reactivity in term labor and threatened preterm delivery

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To obtain data on the immunologic events during labor, we investigated cytotoxic activity, progesterone-prostaglandin $F_{2\alpha}$ and prostaglandin E_2 sensitivity of the lymphocytes in late pregnancy at early and late stages of labor and in the puerperium. In an attempt to investigate common determinants between the behavior of lymphocytes during labor and threatened preterm delivery, the same parameters of lymphocytes obtained from women with threatened preterm delivery were tested in parallel. At the beginning of labor, cytotoxic activity of the lymphocytes significantly increased and remained at a high level till the end of labor. A negative correlation ($r = -0.803$) was found between the increase of cytotoxicity and the duration of labor. High progesterone sensitivity of pregnancy lymphocytes decreased while low sensitivity to prostaglandin E_2 increased during labor. Sensitivity of the lymphocytes to prostaglandin $F_{2\alpha}$ was similar in all of the groups examined. Except for prostaglandin E_2 sensitivity all parameters of the lymphocytes obtained from women with threatened preterm delivery were similar to those of lymphocytes obtained during labor. (AM J OBSTET GYNECOL 1986;155:108-12.)

Key words: Cytotoxicity, prostaglandin, progesterone

The etiology of preterm deliveries and spontaneous abortions is still unsolved. A part of preterm deliveries of unknown etiology certainly has an immunologic background. There are data showing that in vitro reactivity of pregnancy lymphocytes is significantly lower than that of lymphocytes from nonpregnant women.¹⁻³ On the other hand, lymphocytes of some women with threatened preterm delivery show a significantly higher cytotoxic activity than lymphocytes of healthy pregnant women.⁴ We have previous data showing that cytotoxic activity of the lymphocytes increases during labor and that lymphocytes of women with threatened preterm delivery act similarly to those of healthy pregnant women obtained during labor.⁵

Therefore we think it is worthwhile to investigate immunologic phenomena during labor and to look for common denominators between labor and preterm delivery. Better understanding of the mechanism of delivery might provide new information on the immunologic events in threatened preterm labor.

Material and methods

Patients. Heparinized venous blood was obtained from 25 women in late pregnancy (37 to 40 weeks)

before the onset of labor, at early labor (cervical dilatation was <4 cm), at late labor (cervical dilatation was 5 to 8 cm), and in the puerperium. Lymphocytes were also obtained from 10 women with clinical diagnosis of threatened preterm delivery of unknown etiology. The diagnosis of threatened preterm delivery was based on clinical symptoms such as cervical dilatation and regular uterine contractions between gestational weeks 28 and 37. No anatomic or genetic background of the clinical symptoms could be verified.

Mononuclear cells were separated on Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden) and tested for cytotoxic activity to human embryonic fibroblast target cells. Progesterone (Steraloids Inc., Wilton, Connecticut) was added to the cytotoxicity system in concentrations of 50 to 400×10^{-9} mol/L, prostaglandin E_2 (Sigma, St. Louis) in concentrations of 10^{-6} and 10^{-10} mol/L, and prostaglandin $F_{2\alpha}$ (Enzaprost, Chinoin Pharmaceutical Works, Budapest) in concentrations of 10^{-6} and 10^{-10} mol/L.

A cytotoxicity test based on the assessment of endogenous alkaline phosphatase activity of the target cells was used.⁶ By this test natural killer activity is determined.⁷ The assay was performed as follows: human embryonic fibroblast cells derived from 10- to 12-week-old embryos were used as targets. Nunclon tissue culture plates were seeded with 5000 target cells per well suspended in 0.2 ml of medium 199 supplemented with 10% fetal calf serum. The target cells were allowed to attach by overnight incubation. The following day the

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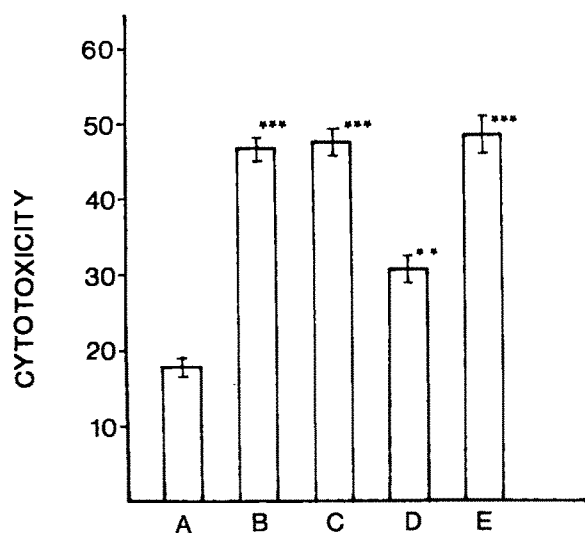


Fig. 1. Cytotoxic activity of the lymphocytes in late pregnancy (A), in early labor (B), in late labor (C), and in the puerperium (D), as well as that of the lymphocytes obtained from women with threatened preterm delivery (E). Columns A, B, C, and D represent mean $\pm$ SEM of 25 tests; column E represents mean $\pm$ SEM of 10 tests. ** = Significantly different from the control (A) at $p < 0.01$. *** = Significantly different from the control (A) at $p < 0.001$.

medium was replaced by 0.2 ml of lymphocyte suspension containing 5×10^5 lymphocytes in 0.2 ml of the same medium. After 16 hours of incubation, lymphocytes and damaged target cells were removed by repeated washing and the substrate of alkaline phosphatase was added to the surviving target cells. The amount of the yellow reaction product was determined photometrically at 405 nm. The percentage reduction in enzyme activity of the test compared to the target cell control was regarded as an indicator of cytotoxicity.

Statistical analysis. The two-tailed Student's t test and regression analysis were used for evaluation of the data.

Results

Changes of cytotoxic activity of the lymphocytes during labor. Cytotoxic activity of the lymphocytes during labor and that of the lymphocytes obtained from women with threatened preterm delivery was significantly higher than that of the lymphocytes obtained in late pregnancy (Fig. 1). Furthermore, inverse relationship ($r = -0.803$) was found between the increase of cytotoxicity and the duration of labor (Fig. 2).

Sensitivity of the lymphocytes to progesterone and prostaglandin during pregnancy, labor, and threatened preterm delivery. (1) Cytotoxic activity of the lymphocytes was determined in the presence of progesterone (0.5 , 2 , and 4×10^{-7} mol/L). In case of lymphocytes obtained in late pregnancy 0.5×10^{-7} mol/L of progesterone inhibited cytotoxicity by 50%. Lym-

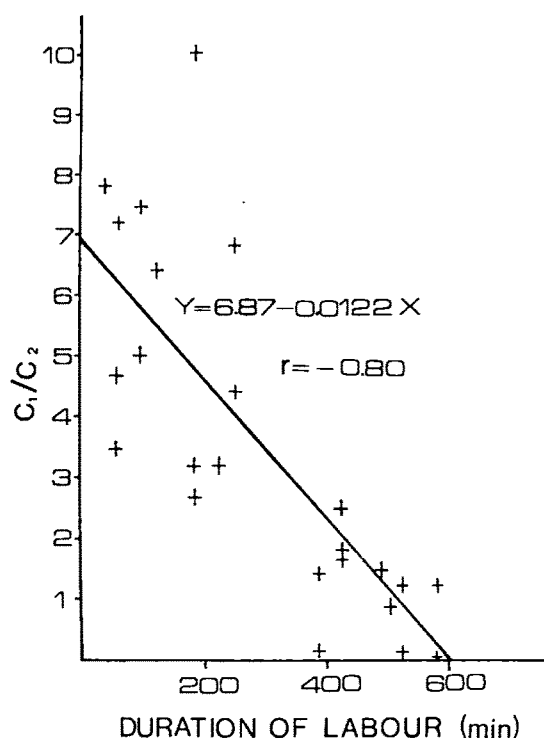


Fig. 2. The relationship between changes of cytotoxic activity of the lymphocytes and the duration of labor. C_1 = Cytotoxic activity of the lymphocytes in late labor. C_2 = Cytotoxic activity of the lymphocytes in late pregnancy.

phocytes obtained in early or late labor and in the puerperium were less sensitive to the suppressive effect of progesterone (2.5×10^{-7} mol/L, 4×10^{-7} mol/L, and 2×10^{-7} mol/L of progesterone, respectively, was required for 50% inhibition of cytotoxicity) (Fig. 3). Progesterone sensitivity of the lymphocytes obtained from women with threatened preterm delivery was tested previously and found to be even smaller than that of the lymphocytes during labor. (2) A different trend was observed by prostaglandin E_2 treatment of the lymphocytes. Incubation of late pregnancy lymphocytes with prostaglandin E_2 at a concentration of 10^{-6} mol/L caused 48% inhibition of cytotoxicity, 10^{-10} mol/L of prostaglandin E_2 did not have significant effect on cytotoxicity. In early labor sensitivity of the lymphocytes to the inhibitory effect of prostaglandin E_2 significantly increased. Cytotoxic activity was reduced by nearly 90% when lymphocytes were treated with 10^{-6} mol/L of prostaglandin E_2 and the substance caused a 70% inhibition even at a concentration of 10^{-10} mol/L. At delivery (though to a somewhat smaller extent) prostaglandin E_2 still markedly inhibited lymphocyte cytotoxicity, and in the puerperium sensitivity to prostaglandin E_2 was still higher than that at the end of pregnancy. Lymphocytes of women with threatened preterm delivery were less sensitive to the blocking effect of prostaglandin E_2 than those obtained during

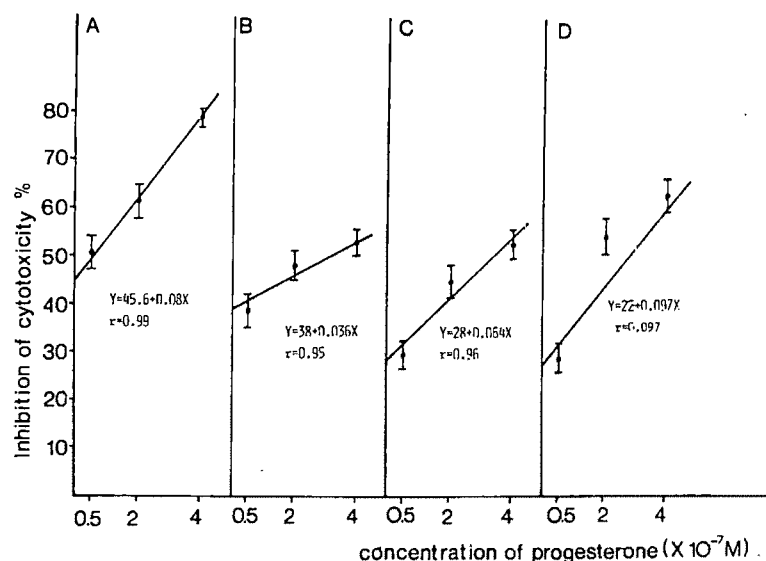


Fig. 3. Progesterone sensitivity of the lymphocytes in late pregnancy (A), in early labor (B), in late labor (C), and in the puerperium (D). Each point of the curve represents mean $\pm$ SEM of 25 separate tests.

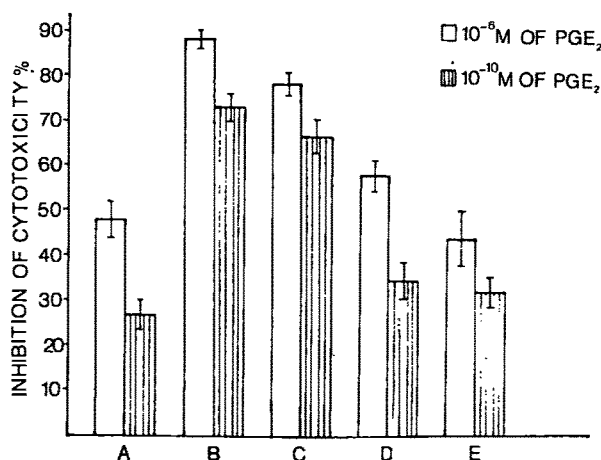


Fig. 4. Prostaglandin E₂ sensitivity of the lymphocytes in late pregnancy (A), in early labor (B), in late labor (C), and in the puerperium (D), as well as that of the lymphocytes obtained from women with threatened preterm delivery (E). Columns A, B, C, and D represent mean $\pm$ SEM of 25 tests; column E represents mean $\pm$ SEM of 10 tests.

labor. Even 10⁻⁶ mol/L of prostaglandin E₂ was not able to cause 50% reduction of cytotoxicity (Fig. 4). (3) When lymphocytes were treated with 10⁻⁶ mol/L of prostaglandin F_{2a} cytotoxicity significantly increased in all of the stages examined. Prostaglandin F_{2a} in a concentration of 10⁻¹⁰ mol/L did not cause any significant change of cytotoxicity. We did not find marked difference in prostaglandin F_{2a} sensitivity of the lymphocytes of either of the groups examined (Fig. 5).

Comment

The survival of the immunologically incompatible fetus in a hostile maternal environment is partly ex-

plained by the suppression of maternal immune reactivity.^{8,9}

Based on this finding we assume that alteration of lymphocyte responsiveness plays a role in the initiation of labor, so one would expect an increase in immune reactivity at delivery. Our data support this assumption. They allow the conclusion that there is a relationship between lymphocyte cytotoxicity and labor. This hypothesis is supported by two findings: (1) The low cytotoxic activity of pregnancy lymphocytes significantly increased by the beginning of labor and remained high till delivery. (2) Inverse relationship was found between the increase of cytotoxicity and the duration of labor.

To obtain a more complex view on the changes of lymphocyte reactivity by labor we investigated the progesterone and prostaglandin sensitivity of the lymphocytes. High progesterone sensitivity of pregnancy lymphocytes significantly decreased, whereas low prostaglandin E₂ sensitivity increased during labor. There was no marked difference in sensitivity to the stimulatory effect of prostaglandin F_{2a} between lymphocytes obtained in late pregnancy and those obtained during labor.

Sensitivity of the lymphocytes to prostaglandin E₂ (suppressive to cytotoxicity) increased, whereas their sensitivity to prostaglandin F_{2a} practically did not change during labor, but their cytotoxic activity became higher. There are more possible explanations for this apparent contradiction.

One is that the effect of a certain compound does not only depend on the sensitivity of the target cell but also on the amount of the compound available. Although lymphocyte sensitivity to prostaglandin F_{2a} did

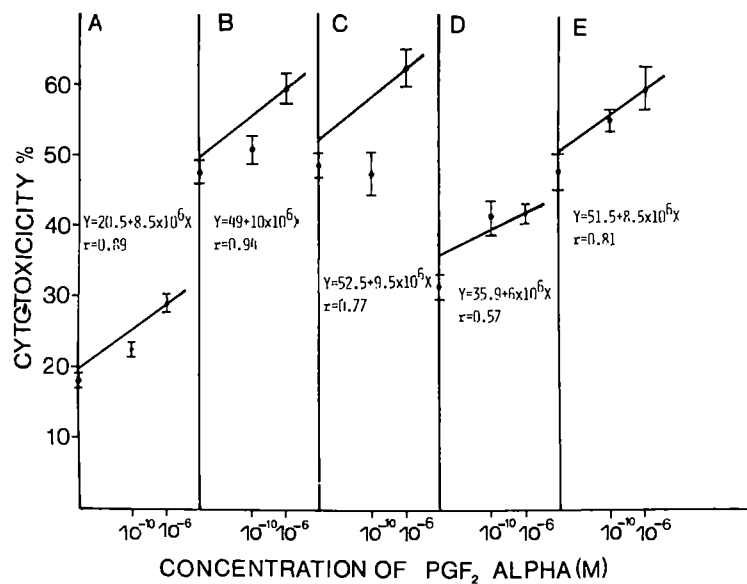


Fig. 5. Prostaglandin F_{2α} sensitivity of the lymphocytes in late pregnancy (A), in early labor (B), in late labor (C), and in the puerperium (D), as well as that of the lymphocytes obtained from women with threatened preterm delivery (E). Each point of the curve represents mean ± SEM of 25 (in the case of A, B, C, and D) or 10 (in the case of E) separate tests.

not change during labor and sensitivity to prostaglandin E₂ increased, the proportion of the two compounds changes during labor,¹⁰ and the prostaglandin E₂/prostaglandin F_{2α} ratio, which is about 1 in term serum, gives a value of 0.3 during labor.¹¹ So, probably the amount of prostaglandin F_{2α} that was not high enough to cause any change in lymphocyte reactivity during pregnancy becomes sufficient by labor to influence cytotoxic activity of the lymphocytes. Reduction of progesterone sensitivity might also contribute to the final result, since as we have previously shown progesterone concentration present in third-trimester pregnancy sera is sufficient to cause significant reduction in *in vitro* reactivity of pregnancy lymphocytes but not in reactivity of lymphocytes of nonpregnant women or that of women with threatened preterm delivery.¹²

The pathophysiology of certain types of spontaneous abortions or preterm deliveries can probably also be explained by this mechanism. There are certain similarities in the behavior of lymphocytes obtained during labor and those obtained from women with threatened preterm delivery of unknown etiology. One of them is increased cytotoxic activity and the other one is reduced progesterone sensitivity of the lymphocytes compared to those of healthy pregnant women. In a previous paper we have shown that progesterone sensitivity of the lymphocytes obtained from women with threatened preterm delivery is significantly lower than that of healthy pregnant women's lymphocytes.¹² Although to a lesser extent, lymphocytes obtained during labor also tend to become hyporesponsive to progesterone as compared to those obtained in late pregnancy.

Sensitivity of the lymphocytes to the stimulatory effect of prostaglandin F_{2α} was similar in all of the groups examined, whereas sensitivity to prostaglandin E₂ was lower in the lymphocytes obtained from women with threatened preterm delivery than in that of the lymphocytes obtained during labor. This sensitivity as well as the higher prostaglandin E₂/prostaglandin F_{2α} ratio in the serum are the only parameters different from those observed during delivery. Despite the relatively high concentration of prostaglandin E₂ in the serum, cytotoxicity of the lymphocytes is high, which can be explained by the low sensitivity of the lymphocytes to the blocking effect of prostaglandin E₂ and by the fact that they are irresponsive to the blocking effect of serum progesterone.

In conclusion, we can say that the termination of pregnancy (term and preterm labor) is accompanied by an increase of cytotoxic activity of the lymphocytes. Whether there is a cause-effect relationship between increased cytotoxicity and the initiation of labor still remains a question; however, the fact that there is positive correlation between the change of cytotoxicity and the duration of labor supports this assumption. The events leading to increased cytotoxicity are probably multifactorial, that is, (among others) progesterone sensitivity, prostaglandin sensitivity and serum concentrations of these compounds all might play a role. Lymphocytes of women with threatened preterm delivery of unknown etiology behave similarly to lymphocytes obtained during delivery, so probably manipulation of these parameters might have a beneficial effect in these cases on the outcome of pregnancy.

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Programmed oocyte retrieval during routine laparoscopy and embryo cryopreservation for later transfer

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Fixed-schedule ovulation induction and cryopreservation of the obtained embryos was performed in women undergoing a preliminary laparoscopy for infertility investigation before possible inclusion in an in vitro fertilization program. The cycle before follicular stimulation was modified by a progestogen or an estrogen-progestogen contraceptive pill. Ovarian inaccessibility precluded follicular aspiration in four of 34 patients but at least one oocyte was obtained in 29 of the remaining 30. Although fewer oocytes were obtained in these patients than in a control group undergoing in vitro fertilization treatment, one or more embryos were obtained in 22 patients in the study group. All embryos were frozen and to date 25 embryos from 17 patients have been thawed. Embryos have been placed in 16 of the 17 patients and six pregnancies have been initiated. Three are currently ongoing, one ectopic pregnancy was recorded, and two pregnancies were classified as "chemical." Programmed oocyte retrieval and embryo cryopreservation resulted in an extra chance of pregnancy in patients undergoing a laparoscopy for infertility investigation. (*AM J OBSTET GYNECOL* 1986;155:112-7.)

Key words: Programmed oocyte retrieval, embryo cryopreservation, in vitro fertilization, laparoscopy for infertility

An acceptable recovery rate of preovulatory eggs can be obtained with the use of a fixed schedule of ovulation induction and follicular aspiration.^{1,2} We have investi-

gated this modality of treatment in volunteers undergoing routine laparoscopy for infertility investigation. When embryos were obtained, we decided to freeze them according to a particular cryopreservation technique³ to permit transfer to be delayed until a later cycle. Immediate transfer was excluded on account of the necessary uterine and tubal manipulations that were performed during the laparoscopy (hysteroscopy, tubal hydrotubation, or endometrial biopsy). In this communication, we report the methods used, the preliminary data from Programmed Oocyte Retrieval and

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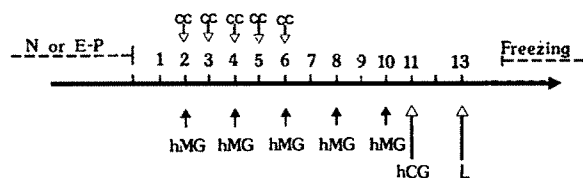


Fig. 1. Programmed oocyte retrieval and embryo cryopreservation treatment schedule. The numbers represent the cycle days. L = Laparoscopy.

Embryo Cryopreservation (P.O.R.E.C), and the first pregnancies obtained after transfer of thawed embryos.

Patients and methods

Between April and September, 1985, 34 patients with long-standing infertility were recruited from our gynecologic clinics. Each couple was fully informed and agreed to the procedure. The indication for laparoscopy was a morphologic examination of the pelvis in infertile women with known pelvic abnormality ($n = 24$) or for long-term (>5 years) idiopathic infertility ($n = 10$).

The date of the laparoscopy was decided several weeks in advance (between 5 and 9 weeks) depending on operating theater schedules, the dates of the patients' menstrual periods, and the patients' availability.

The preceding cycle was modified by the daily administration of an estrogen-progestogen contraceptive tablet (group E-P) (Triella, Cilag, Paris, France), usually from the fifth day of the cycle ($n = 21$), or norethindrone (group N) (Norluten, Smith, Kline & French, Paris, France), 10 mg/day ($n = 13$). In group N, four patients commenced treatment on the fifth day of the cycle (for 23 and 28 days) and nine on day 15 in the luteal phase (for 10 to 17 days). The day of stopping the treatment was arbitrarily considered as day 0 and the following day as the first day of the stimulated cycle (Fig. 1).

Follicular stimulation was achieved by a combination of 100 mg of clomiphene citrate (Clomid, Merrel Toussaint, Paris, France) from day 2 to day 6 and 2 ampules per 24 hours of human menopausal gonadotropin (hMG) (Inductor, Searle, Paris, France) every other day from day 2 to day 10 inclusive. Ovulation induction was systematically triggered on day 11 by the injection of 5000 IU of human chorionic gonadotropin (hCG, Organon, St. Denis, France) without prior knowledge of the level of estradiol and luteinizing hormone (the blood sample having been taken at the time of hCG injection and the results only being available the following day). There was no echographic monitoring of the size of the follicles. Oocyte recovery was performed between 34 and 37 hours after the administration of hCG. The process of oocyte recovery and in vitro culture was as previously described in our in vitro fertilization unit.⁴

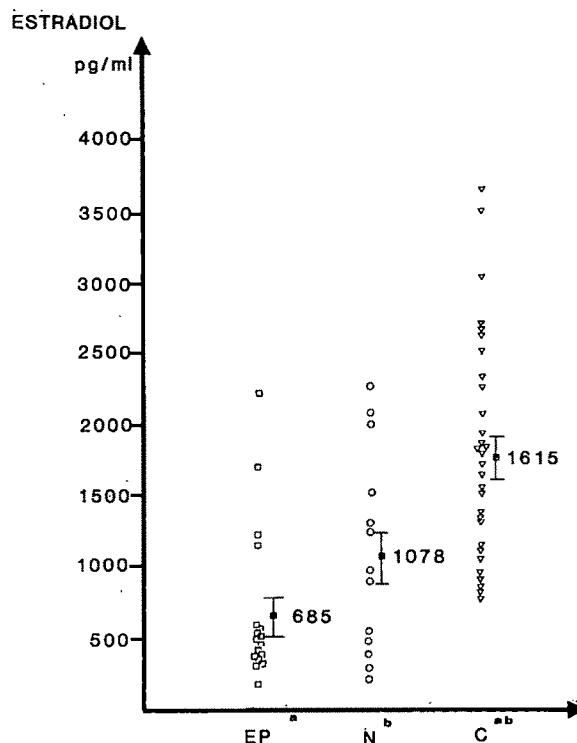


Fig. 2. Serum estradiol levels at the time of hCG injection in the three groups. Mean $\pm$ SEM are shown. a and b = $p < 0.01$.

Cryopreservation was usually performed with 1- or 2-day-old embryos with propylene glycol used as a cryoprotectant as recently described.^{3,5} These embryos remain in a state of cryopreservation before thawing and transfer in a later natural cycle, 2 to 4 days after the serum luteinizing hormone peak, depending on the stage of cryopreservation.⁵ The transfers were performed between one and six cycles after laparoscopy. Those patients whose tubes were not unequivocally occluded were recommended to abstain from sexual intercourse during the transfer cycle.

A control group (group C) was randomly selected from women undergoing laparoscopic oocyte recovery during the period of this study. This group differed from the program patients in that they did not receive a progestogen or an estrogen-progestogen before the laparoscopy cycle. They were given the same form of follicular stimulation of clomiphene citrate and hMG, hCG was not given in a fixed schedule but was withheld until follicular development was thought to be optimal by our conventional monitoring techniques.⁴

Mean values and SEM were compared with the use of Student's t test and the various proportions were compared by means of the χ^2 test.

Results

In four cases, follicular aspiration was not possible because of the inaccessibility of the ovaries. Thirty pa-

Table I. Follicular stimulation oocyte recovery and cleavage rate in the control group (group C) and the two program groups (groups N and EP)

	Group C (n = 30)	Group N (n = 13)	Group EP (n = 17)
Estradiol (pg/ml) (mean $\pm$ SEM)	1615 $\pm$ 147*†	1078 $\pm$ 192*	685 $\pm$ 141†
Follicular volume (ml) (mean $\pm$ SEM)	4.43 $\pm$ 0.32‡	4.48 $\pm$ 0.88§	2.28 $\pm$ 0.52‡§
Follicles aspirated (n)	143	42	47
Oocytes recovered (n)	110 (77%)	32 (76%)	30 (64%)
Embryos (n)	67 (61%)	17 (53%)	16 (53%)

*p < 0.05.

†p < 0.001.

‡p < 0.001.

§p < 0.001.

Table II. Results by patient in the control group (group C) and the two program groups (groups N and EP)

	Group C (n = 30)	Group N (n = 13)	Group EP (n = 17)
Follicles per patient	4.77 $\pm$ 0.32*†	3.23 $\pm$ 0.28*	2.80 $\pm$ 0.36†
Oocytes per patient	3.67 $\pm$ 0.37‡§	2.46 $\pm$ 0.31‡	1.76 $\pm$ 0.28§
Embryos per patient	2.23 $\pm$ 0.32	1.30 $\pm$ 0.33	0.88 $\pm$ 0.17

*p < 0.01.

†p < 0.001.

‡p < 0.05.

§p < 0.01.

||p < 0.01.

tients therefore underwent follicular puncture in the program group and 30 procedures were performed in the control group. Seventeen received estrogen-progestogen contraceptive (group E-P) and 13 received norethindrone (group N).

The 30 programmed laparoscopies were performed arbitrarily on day 13. The day was later than that for control group C (mean = 12.2 $\pm$ 0.2 p < 0.001) because the slowing of follicular maturation provoked by the programming had been taken into account.⁶

As shown in Fig. 2 the plasma estradiol level at the time of hCG injection was significantly higher (p < 0.01) in the control group (group C) (mean = 1615 $\pm$ 136 pg/ml) than in the two groups of patients whose laparoscopies were programmed (group N, mean = 1078 $\pm$ 198 pg/ml; group E-P, mean = 685 $\pm$ 141 pg/ml). The serum estradiol level in the group programmed with estrogen-progestogen contraceptive tablets demonstrated a clear trend to be lower than in the group programmed with norethindrone but a significant difference between the two groups was not demonstrable.

The luteinizing hormone level was <10 mIU/ml at the time of hCG injection in all but six of the 30 patients. We must presume that for these six patients the hCG

injection was performed just after the beginning of spontaneous luteinizing hormone secretion since oocytes were recovered from the follicles punctured 34 to 37 hours later for each one of these six patients.

The mean volume of follicular fluid aspirated was not different in groups N and C (4.48 $\pm$ 0.88 ml per follicle in group N compared with 4.43 $\pm$ 0.32 ml per follicle in group C) but was significantly lower in group E-P (2.28 $\pm$ 0.32 ml per follicle) (Table I).

The same number of follicles per woman was obtained in groups E-P and N (Table II) but both values were significantly lower than in group C (2.80 $\pm$ 0.36, 3.23 $\pm$ 0.28, and 4.77 $\pm$ 0.32, respectively).

In all of the programmed laparoscopies in which follicular aspiration was performed (n = 30), at least one oocyte was obtained except in one case in group E-P. Thirty-two oocytes were recovered from the follicles aspirated in group N and 30 in group E-P, compared with 110 in group C (Table I). The percentage of oocytes per follicle was identical in groups N (76%) and C (77%) and not significantly higher than that of group E-P (63%) (Table I). When the number of oocytes per woman was compared, there was a slight but nonsignificant difference between group N (2.46 $\pm$ 0.31 oocytes per woman) and group E-P (1.76 $\pm$ 0.28). Both

Table III. Survival of thawed embryos after programmed oocyte retrieval and embryo cryopreservation

Freezing stage	No. of embryos	Cells surviving after thawing		
		100%	50%-80%	0%-40%
2 Pronuclei	8	8	—	—
2 Cells	1	1	—	—
≤4 Cells	9	6	2	1
>4 Cells	7	1	2	4

Table IV. Analysis of the six pregnancies

Patient No.	Age (yr)	Program group	Estradiol (pg/ml)	Oocytes inseminated (n)	Embryos frozen (n)	Embryos transferred (n)	Type of infertility	Duration of infertility	Pregnancy outcome
1	28	EP	330	2	2	2	Damaged tubes	6	Chemical
2	30	EP	480	4	2	2	Damaged tubes	5	Ongoing (7 wk)
3	36	N	545	3	3	3	Idiopathic	7	Chemical
4	32	EP	585	3	1	1	Idiopathic*	9	Ongoing (26 wk)
5	33	N	1480	2	2	2	Blocked tubes	3	Ongoing (15 wk)
6	32	EP	1700	1	1	1	Damaged tubes†	6	Ectopic

*Sexual intercourse 7 days before embryo transfer.

†Sexual intercourse 3 days before embryo transfer.

had a significantly lower number than in the control group ($p < 0.05$ and $p < 0.001$) (Table II).

The cleavage rates of the recovered oocytes were 53% in group N, 53% in group E-P, and 61% in group C. This difference is not significant. The percentages of immature or atretic oocytes were similar in all three groups.

The number of embryos obtained per patient was significantly lower in group E-P (0.88 ± 0.17) than in group C (2.32 ± 0.32) ($p < 0.01$). Ten of the 13 patients in group N (77%) and 12 of the 17 patients in group E-P (71%) had at least one embryo.

Thirty-three embryos (17 from 10 patients in group N and 16 from 12 patients in group E-P) were frozen 1 or 2 days after in vitro insemination in order to be thawed and transferred in later natural cycles. Up to the present, 25 of the 33 frozen embryos have been thawed. Twenty of the 25 thawed embryos (80%) survived with half or more of their blastomeres intact. This proportion rises if we consider the embryos at the two-pronuclei to four-cell stage (Table III). In this group 17 of the 18 thawed embryos survived (94%) and 15 (83%) were totally intact.

The embryos of 17 patients have been thawed to date. Twenty-four embryos have been transferred into 16 patients one to six cycles after the laparoscopy. Among these patients, six pregnancies occurred (two "chemical," one ectopic, and three ongoing) (Table IV). The pregnancy rate of this method is therefore 38% after embryo transfer. The ongoing pregnancy rate

Table V. Summary of patient outcome in P.O.R.E.C.

	n	%
Patients in P.O.R.E.C. trial	34	
Patients with		
≥1 follicle aspirated	30	
≥1 oocyte recovered	29	97*
≥1 embryo frozen	22	73*
≥1 embryo thawed to date	17	
≥1 embryo transferred	16	94†
Pregnancies initiated	6	38‡
Ongoing pregnancies	3	19‡

*Per patient undergoing follicular aspiration.

†Per patient having thawed embryos.

‡Per embryo transfer.

is 19% in the patients receiving embryo transfers (Table V).

Comment

The importance of laparoscopy before in vitro fertilization is controversial. Some groups⁷ propose it as advantageous as it permits the confirmation of the indication for in vitro fertilization and the verification of ovarian accessibility. Others⁸ regard it as excessive as it involves submitting the patient to a combined anesthetic and surgical risk without being directly therapeutic. We have always believed this examination to be indispensable. Regardless of whether oocyte retrieval is to be performed by laparoscopy or ultrasound tech-

niques, an accurate knowledge of the current anatomic relationships of the pelvic structures, particularly with reference to the bowel, is essential. Our experience with P.O.R.E.C reinforces this view because, when routine laparoscopy for infertility investigations is necessary, it is now possible to perform a complete gynecologic surgical assessment of the pelvis (tubal hydrotubation, endometrial biopsy, and, if necessary, hysteroscopy) and at the same procedure give to the patient an extra chance of pregnancy at least equal to the pregnancy rate per transfer with classical in vitro fertilization treatment obtained in the leading centers. This work leads us to suggest that all preliminary laparoscopies in patients being considered for in vitro fertilization should be performed in the in vitro fertilization center (if it is capable of embryo cryopreservation) rather than in the local referral center.

In the report of Templeton et al.³ of fixed schedule ovulation induction, a preference was expressed for progestogens rather than the combined contraceptive pill to modify the preceding cycle, as at least one cleaving egg was obtained in only 38% of patients receiving the pill compared to 63% with progestogens. In our series the percentage of women undergoing follicular aspiration in whom at least one embryo has been obtained is similar in both groups (71% in group E-P and 77% in group N). The estrogen-progestogen pill does, however, result in reduced levels of serum estradiol in the preovulatory phase that are presumably associated with suppression of the hypothalamic-pituitary-ovarian axis. This reduction does not reach significance, possibly because of the small number assessed.

Although some patients in this protocol received an estrogen-progestogen pill in the cycle preceding embryo transfer, there is no evidence that prior use of oral contraceptives alters the risk of chromosomally abnormal offspring.⁹ Furthermore, in studies of the first postcontraceptive cycle, the only abnormality observed has been a prolongation of the follicular phase.⁶ As four of the six pregnancies resulted from embryo transfer in the first cycle after oocyte retrieval, it is probable that no significant derangement of the cycle is induced by the programming but further investigations on this point are needed.

Follicular maturity is often estimated by the level of plasma estradiol related to the size of the follicles as measured by ultrasound (400 pg/ml of estradiol per follicle >16 mm).¹⁰ Several opinions have been advanced on the relationship between plasma estradiol, oocyte recovery, embryo development, and the occurrence of a pregnancy. Some¹¹ affirm that there is a direct relationship between estradiol and oocyte numbers while others think that the duration of the follicular phase¹² or the speed of evolution of the estradiol curve¹³ are more important criteria than absolute val-

ues. Nevertheless, a variable but significant decrease in oocyte recovery on account of poor ovarian response is reported.¹⁴ We believe this attitude merits reanalysis. In particular, when we look at the six patients in whom pregnancy occurred in this series, there appears to be no relationship between follicular steroidogenic activity as reflected in serum estradiol levels, embryonic yield, and developmental potential (Table IV).

It is apparent that fewer embryos were obtained in the program patients than in the control groups. This was related to the diminished number of oocytes aspirated as the cleavage rates were similar in both groups. However, it is not possible to state whether this is due to the programming itself or to other practical considerations because the three groups are not strictly comparable. The program patients were by definition undergoing preliminary laparoscopy and, although the four patients with inaccessible ovaries have not been included in the tabulation of results, there were other patients in whom ovarian access, although possible, was severely limited by adhesion formation. The control group, however, had all been previously selected for laparoscopic oocyte retrieval on the basis of ovarian accessibility determined at the time of the preliminary laparoscopy. Furthermore, the programmed laparoscopies were performed in the operating theater block without knowledge of the number of oocytes harvested whereas the control group underwent follicular aspiration in the in vitro fertilization theater in direct communication with the embryology laboratory and the techniques of follicular aspiration could be modified if the oocyte yield was low.

At least one oocyte was obtained in 29 of 30 patients who underwent follicular puncture in the program group, and 22 of these 30 patients (73%) had at least one embryo available for freezing. It has been suggested that the potential for embryo implantation is enhanced if the selection of embryos for freezing is based on their ability to attain certain developmental stages before freezing.¹⁵ In the present study all embryos obtained from the program patients were frozen and there was no selection of embryo quality apart from the obligatory exclusion of polyspermic eggs. Nevertheless this group of embryos proved to be remarkably resistant to the freezing-thawing procedure. It is thought that the improved implantation rate of cryopreserved embryos in comparison with fresh embryos is due to replacement in the natural cycle and the fact that embryos that are best able to withstand the cryopreservation procedures are the "better quality" embryos.¹⁶ Although there are not sufficient data for confirmation, it is possible that programming of oocyte recovery helps to select a group of "healthy" oocytes that are able to achieve maturity despite prior suppression of ovarian function. This is suggested by the fact

that survival after cryopreservation and thawing of the embryos resulting from insemination of these programmed oocytes is at least equal to the overall survival of frozen-thawed embryos in our unit. The pregnancy rates per transfer are similar for programmed and non-programmed cryopreserved embryos.⁵

The programming of oocyte retrieval and embryo cryopreservation for later transfer (P.O.R.E.C) gives an extra chance of pregnancy that appears to be at least equivalent to the success rate of in vitro fertilization in the leading centers in patients in whom a laparoscopy is performed for the investigation of infertility. The preliminary results of our trial prompt us to continue with this technique, which should be able to be improved by modification of the follicular stimulation regimens to allow the recovery of more oocytes. In the light of our experience we have decided to extend programmed oocyte retrieval to include in vitro fertilization treatment cycles.

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Cortisol levels in human pregnancy in relation to parity and age

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Data are presented of a study on the relationship between cortisol, amenorrhea, parity, and age among pregnant women. Total cortisol concentrations were measured by a radioimmunoassay in healthy pregnant women ($n = 527$), including 105 nulliparas and 422 multiparas. The serum concentration of total cortisol increased linearly with progression of amenorrhea. Significantly higher cortisol levels were found in nulliparas compared to multiparas and independent of amenorrhea. Since parity and age are strongly correlated, age-dependent changes may contribute to the difference in cortisol level between nulliparas and multiparas. Analysis of the age-dependent changes on this difference in cortisol level showed no significant influence. (AM J OBSTET GYNECOL 1986;155:118-21.)

Key words: Cortisol, pregnancy, parity, age

It has been reported repeatedly that the plasma concentration of cortisol, measured as the total amount of both bound and unbound cortisol, increases during pregnancy,^{1,2} though the absolute cortisol values reported in these studies varied considerably.

In all studies an early cortisol increase around the seventh to tenth week of gestation was noted, whereas the results of some studies suggested a progressive increase till the end of the second trimester (weeks 26 to 32) and maintenance at that level until labor commences.¹ In none of these studies was the limited amount of data tested for linearity, and parity was not considered as a variable in those analyses. Nolten and Rueckert³ observed higher concentrations of free cortisol during pregnancy of primigravidas than did authors who studied overall data from both primigravid and multigravid women.²

In view of the well-known immunosuppressive properties of cortisol, the raised cortisol levels during pregnancy might be related to an increased prevalence and morbidity of some diseases, for example, malaria,⁴ in the first compared to subsequent pregnancies. This prompted analysis of the serum concentration of cortisol during pregnancy in relation to parity. Since parity is strongly related to age and since age-dependent

changes in serum cortisol level have been described,⁴ age was included as a parameter in the analysis.

Material and methods

The study group consisted of 527 healthy pregnant women, 105 nulliparas and 422 multiparas, who repeatedly visited the antenatal clinics of Turiani Hospital, Turiani, Tanzania, in the period of February 1981 to May 1982. Only one sample was taken from each woman during her visit to the clinic.

The amenorrheal period was derived from the fundal height and the anamnestic amenorrhea and recorded in an even number of weeks.

Since the serum cortisol concentration exhibits diurnal variation, all blood samples collected by venous puncture were obtained between 11 AM and 1 PM, while patients were in a sitting position. Blood samples were stored in a refrigerator for about 2 hours and then centrifuged (10,000 rpm) for 10 minutes. Serum was separated and stored at -20°C , transported by air in a frozen condition to Nijmegen, The Netherlands, and again stored at -20°C until analyzed.

Total serum cortisol concentration was determined with the Autopak cortisol test kit system (Micro-Medic Systems). The test consists of a quantitative radioimmunoassay for the determination of cortisol in human serum, with use of cortisol-iodine 125-histamine as tracer. Rabbit antibodies raised against cortisol were fixed to test-tubes as the solid phase. The tests were run in the fully automated Concept-4 (Micro-Medic Systems). The sensitivity of this radioimmunoassay was $0.1\text{ }\mu\text{g/dl}$ at 95% actual binding/initial binding. The interassay coefficient of variation was determined with five control sera, 0.18 ± 0.01 , 0.28 ± 0.01 , 0.37 ± 0.02 , 0.76 ± 0.04 , 0.91 ± 0.03 (mean $\pm$ standard de-

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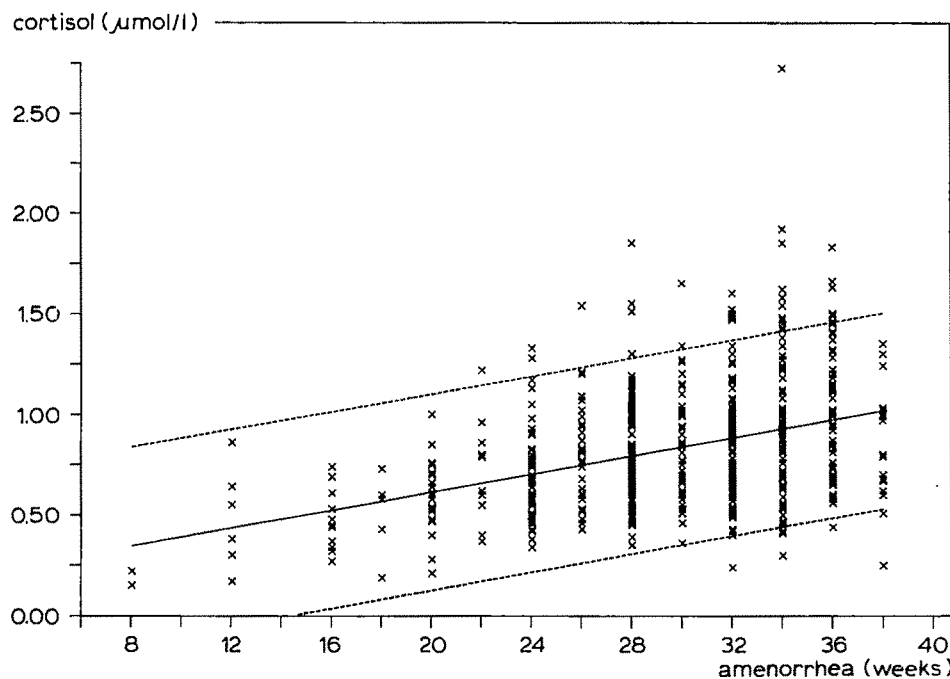


Fig. 1. Serum concentration of total cortisol in healthy pregnant women ($n = 527$); regression line and 90% prediction curves are given.

Table I. Number of samples from nulliparous and multiparous women in relation to amenorrhea

	Amenorrhea (wk)															
	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38
Nulliparas	0	0	4	0	3	0	8	1	17	9	15	10	13	12	10	3
Multiparas	2	0	4	0	8	5	12	9	32	22	73	35	75	74	56	15

viation), included in 14 series of measurements. The coefficient of variation was 6.8%, 3.8%, 5.8%, and 3.7%, respectively. Since the assayed samples covered a broad range of values, the intraassay coefficient of variation was calculated with the double deviation of 25 samples for the five consecutive ranges (0.07 to 0.28, 0.28 to 0.55, 0.55 to 1.11, 1.11 to 1.66, and 1.66 to 2.22 $\mu\text{mol/L}$) with their coefficient of variation being 3.4%, 4.8%, 4.5%, 4.7%, and 4.3%, respectively. The overall intraassay coefficient of variation was 4.5%. All the measurements were made with a maximum binding of $27.8\% \pm 5.0\%$ (mean $\pm$ SD).

Results

Serum cortisol in relation to amenorrhea. Total cortisol was determined in the sera of 527 pregnant women. The results plotted against amenorrhea are depicted in Fig. 1. Results indicate a steady increase of the total cortisol concentration with increasing amenorrhea. Statistical analysis showed that the mean cortisol concentration for each amenorrheal period was not the same (one-way analysis of variance; $p < 0.001$), while the hypothesis of a linear relation between mean

cortisol and amenorrhea could not be rejected (F test; $p = 0.64$). Hence the data indicate a linear relationship between cortisol and amenorrhea. The regression relation was estimated by $y = 0.17 + 0.022x$, where y = mean cortisol concentration ($\mu\text{mol/L}$) and x = amenorrhea (weeks).

Serum cortisol in relation to parity. Pregnant women were divided in nulliparas and multiparas (Table I). The corresponding serum concentrations of cortisol were plotted against amenorrhea for each group and are depicted in Fig. 2. The estimated regression lines were $y = 0.28 + 0.021x$ for the nulliparas and $y = 0.095 + 0.024x$ for the multiparas. The slopes of both lines were significantly different from zero ($p < 0.001$).

Assuming parallelism of regression lines (parallelism test; $p = 0.61$), a significant difference between nulliparas and multiparas was found ($p = 0.001$); the distance between both lines amounts to 0.11 $\mu\text{mol/L}$.

Serum cortisol in relation to age and pregnancy. Since parity is related to age and the cortisol concentration increases during pregnancy, analysis of the relation between age and cortisol levels in pregnant

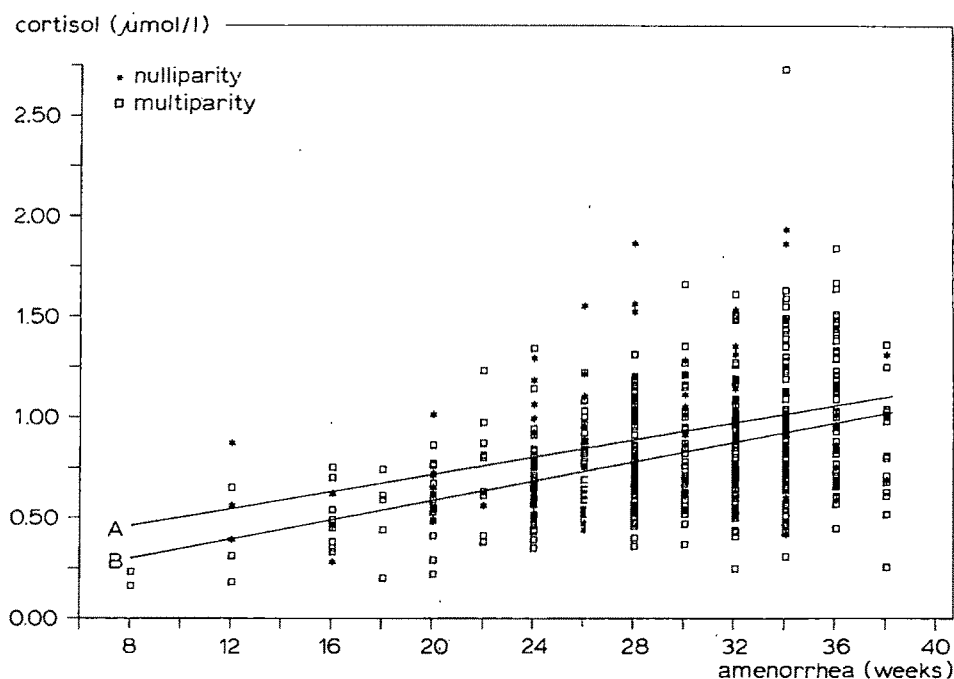


Fig. 2. Serum concentration of total cortisol in healthy pregnant women; comparison between nulliparas ($n = 105$) and multiparas ($n = 422$). Values of total cortisol in nulliparas (*) and multiparas (□) are plotted, and regression lines of nulliparas (A) and multiparas (B) are given.

Table II. Adjusted mean cortisol concentration in relation to age in pregnant women (mean amenorrheal period, 29.6 weeks)

Age group (yr)	Nulliparas		Multiparas	
	n	Mean cortisol concentration (μmol/L)	n	Mean cortisol concentration (μmol/L)
15 ≤ 24	94	0.91	133	0.84
25 ≤ 34	9	0.87	197	0.80
35 ≤ 45	—	—	86	0.78

women must also take into account the factors' parity and amenorrhea.

As a result of incomplete data, only the results of 519 women are considered in this analysis. For the purpose of this analysis both nulliparas and multiparas were subdivided into three arbitrarily chosen age groups: 15 to 24 years, 25 to 34 years, and 35 to 45 years. Among the nulliparas there was no woman in the age group of 35 to 45 years. Amenorrhea was added to this analysis as a covariable factor; a linear relation between amenorrhea and cortisol concentration was presumed, being the same for each parity and age class. Therefore the mean cortisol concentration of each group was recalculated on the basis of an amenorrhea of 29.6 weeks (mean of the total group). The results are given in Table II.

Since parity is correlated to age, the influence of both

factors was analyzed in a two-way analysis of covariance model. When averaged over the lowest age groups, no significant difference between nulliparas and multiparas could be established ($p = 0.17$; interaction test, $p = 0.99$). With regard to the effect of age, the hypothesis that for nulliparous women the means of the age groups 15 to 24 and 25 to 34 years and that for multiparous women the means of all three age groups were the same could not be rejected ($p = 0.40$).

Comment

The serum concentration of pregnant women increased between the eighth and the fortieth week of gestation both in nulliparas as well as in multiparas. Statistical analysis revealed a linear increase of the total serum cortisol concentration in both groups during pregnancy.

Nulliparas have a significantly higher total cortisol concentration than multiparas, and this difference is present throughout pregnancy. This parity effect was not found when the age factor was taken into account. The small decrease in total serum cortisol observed with increasing age was not significant. The difference in the cortisol level of nulliparas and multiparas may be partly caused by age differences; however, the effect of parity on the cortisol level is probably more pronounced than the effect of age.

To our knowledge this is the first description of a parity-dependent serum level of total cortisol.

Conception and the subsequent nidation of the fertilized ovum trigger many hormonal changes that are functional in the fetomaternal interaction. An important aspect of this interaction is the protection of the fetal allograft by limiting the invasive growth of the trophoblast tissue on the one hand and the suppression of immunologic transplantation reactions that may reject the fetal implant on the other.⁵

Several serum factors related to the pregnant state exhibit immunosuppressive properties *in vitro*, such as α -fetoprotein,⁶ human chorionic gonadotropin, human chorionic somatotropin,⁷ progesterone,⁸ estrogens,⁹ or the pregnancy-associated proteins macroglobulin¹⁰ and β -1-glycoprotein.¹¹

In addition, cortisol has well-established immunosuppressive properties.¹² Cortisol can suppress T cell-dependent immune reactions and is related to the inversion of the B/T cell ratio observed during the first trimester of pregnancy.¹³ The considerable increase of the serum cortisol concentration with progressing amenorrhea may be an important factor in pregnancy-associated immunosuppression. This immunosuppression may function in the protection of the fetal allograft, but may possibly be involved as a side effect in the reduced resistance of pregnant women to primary infections or even in loss of established immunity as observed with regard to viral disease, malaria, tuberculosis, and leprosy.¹⁴ The observation that humoral immunity is either unaffected or even enhanced during pregnancy^{7,15} may explain why increased sensitivity to disease is not a general phenomenon during pregnancy. The possible importance of cortisol in this pregnancy-associated immunosuppression is inferred from results observed in experimental murine malaria, showing that loss of a previously existing immunity during pregnancy was related to increased serum corticoid levels and did not occur when increase of the serum concentration was prevented.^{14,16} Meanwhile, a relation between the serum cortisol concentration and malaria during pregnancy has been established in humans also.⁴

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Acute effects of dihydralazine mesylate, furosemide, and metoprolol on maternal hemodynamics in pregnancy-induced hypertension

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The intervillous blood flow and other maternal hemodynamic parameters were assessed in 40 mothers with pregnancy-induced hypertension in late pregnancy before and after hypotensive treatment. Furosemide caused a significant decrease in the intervillous blood flow, whereas the effects of dihydralazine and metoprolol were both negative and positive, that is, more individual and diverse. These changes in the intervillous blood flow did not have any correlation with the changes in other maternal hemodynamic parameters. The importance of placental hemodynamic studies in the evaluation of hypotensive drugs is stressed. (AM J OBSTET GYNECOL 1985;155:122-5.)

Key words: Dihydralazine mesylate, furosemide, metoprolol, intervillous blood flow, pregnancy-induced hypertension

There is general agreement about the maternal indications for hypotensive therapy in pregnancy-induced hypertension because maternal risks are increased only in connection with severe hypertension.

Hypotensive therapy is also used to improve fetal prognosis, even in milder forms of hypertensive disease in many clinics. This policy is based on the idea of increasing intervillous blood flow, which has been shown to be decreased in pregnancy-induced hypertension.^{1,2}

Although a great variety of hypertensive drugs has been used, the ideal drug has not yet been discovered. So far no drug in existence is without complications.

The changes in the intervillous blood flow are of essential importance as far as pregnancy-induced hypertension and medication for it are concerned. In fact, it is impossible to evaluate the appropriateness of hypotensive therapy without knowing the state of the intervillous blood flow and without knowing the effects of medication on it.

The aim of this study was to examine the short-term hemodynamic influences of dihydralazine, furosemide, and metoprolol.

Material and methods

The subjects for the series of tests consisted of 40 mothers in late pregnancy with pregnancy-induced hy-

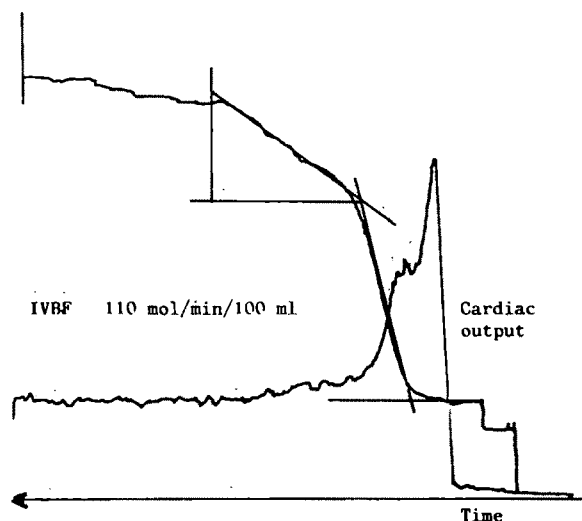


Fig. 1. The cardiac and placental isotope activity curves obtained for the determination of cardiac output and intervillous blood flow (IVBF).

pertension as a complication. The characteristics of the patients are given in Table I. The maternal hemodynamic effects of a single intramuscular dose of 12.5 mg of dihydralazine hydrochloride were tested in 13 mothers. Ten patients were given a single intramuscular dose of 20 mg of furosemide, nine patients were given a single oral dose of 100 mg of metoprolol, while a placebo was used for 8 control subjects. All our patients were volunteers, and the design of the study was approved by the local ethics committee.

The examinations were made in the forenoon at the Department of Physiology in controlled conditions. The maternal heart rate, blood pressure, cardiac out-

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Table I. The characteristics of the patient groups

Patient group	No.	Mean age (yr) $\pm$ 1 SD	Range (yr)	Parity (0/ $\geq$ 1)	Type of pregnancy-induced hypertension (proteinuria/nonproteinuria)	Pregnancy week
Control	8	31.7 $\pm$ 6.0	22-41	5/3	4/4	35.8 $\pm$ 2.9
Hydralazine	11	28.8 $\pm$ 5.8	20-38	7/4	8/3	37.8 $\pm$ 3.0
Furosemide	10	26.2 $\pm$ 4.7	20-36	7/3	5/5	36.0 $\pm$ 3.0
Metoprolol	9	31.4 $\pm$ 8.1	23-43	6/3	6/3	36.7 $\pm$ 3.0

Table II. The correlations (Pearson's correlation) between the drug-induced changes in the intervillous blood flow and other maternal hemodynamic variables in groups receiving different hypotensive medication

Patient group	Change in blood pressure		Change in heart rate	Change in cardiac output	Change in blood volume
	Systolic	Diastolic			
Dihydralazine	-0.31	-0.49	0.26	0.09	0.32
Change in intervillous blood flow	p = 0.18	p = 0.06	p = 0.22	p = 0.40	p = 0.24
Furosemide	-0.11	0.57	0.34	0.19	-0.71
Change in intervillous blood flow	p = 0.38	p = 0.04*	p = 0.24	p = 0.19	p = 0.02*
Metoprolol	-0.26	-0.36	-0.1	0.13	-0.41
Change in intervillous blood flow	p = 0.26	p = 0.18	p = 0.48	p = 0.38	p = 0.14

*Statistically significant.

Table III. The means and standard deviations of the maternal hemodynamic parameters before and after medication

Patient group	Heart rate (L/min)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Cardiac output (L/min)	Blood volume (L)	Intervillous blood flow (ml/min/100 ml)
Control						
Before medication	79 $\pm$ 15	153 $\pm$ 9	98 $\pm$ 8.5	4.9 $\pm$ 1.6	4.9 $\pm$ 0.6	87 $\pm$ 39
After medication	79 $\pm$ 14	152 $\pm$ 15	101 $\pm$ 9	4.7 $\pm$ 1.4	4.8 $\pm$ 0.8	90 $\pm$ 42
	Δ = 0%	Δ = 0%	Δ = 0%	Δ = -4%	Δ = -1%	Δ = 4%
	NS	NS	NS	NS	NS	NS
Dihydralazine						
Before medication	79 $\pm$ 12	153 $\pm$ 18.7	106 $\pm$ 10	7.4 $\pm$ 1.5	6.4 $\pm$ 1.2	67 $\pm$ 29
After medication	96 $\pm$ 16	147 $\pm$ 18.7	94 $\pm$ 15	8.9 $\pm$ 1.9	6.2 $\pm$ 1.4	80 $\pm$ 37
	Δ = 21%	Δ = -4%	Δ = -12%	Δ = 21%	Δ = -3%	Δ = 26%
	p < 0.001	NS	p < 0.01	p < 0.04	NS	NS
Furosemide						
Before medication	83 $\pm$ 14	151 $\pm$ 11	104 $\pm$ 5	7.0 $\pm$ 1.8	5.8 $\pm$ 2.5	86 $\pm$ 35
After medication	80 $\pm$ 13	145 $\pm$ 7	101 $\pm$ 7	6.3 $\pm$ 1.6	5.3 $\pm$ 1.6	68 $\pm$ 22
	Δ = -3%	Δ = -4%	Δ = -2%	Δ = -10%	Δ = -9%	Δ = -21%
	NS	NS	NS	p < 0.02	p < 0.02	p < 0.02
Metoprolol						
Before medication	79 $\pm$ 12	161 $\pm$ 14	108 $\pm$ 12	5.3 $\pm$ 1.9	5.5 $\pm$ 1.9	115 $\pm$ 43
After medication	72 $\pm$ 17	152 $\pm$ 9	101 $\pm$ 7	4.6 $\pm$ 1.5	5.5 $\pm$ 2.0	111 $\pm$ 51
	Δ = -9%	Δ = -6%	Δ = -6%	Δ = -13%	Δ = 0%	Δ = -3%
	p < 0.01	p < 0.01	p < 0.01	p < 0.01	NS	NS

put, blood volume, and intervillous blood flow were determined before and 3 hours after medication in the left lateral recumbent position. Intervillous blood flow was determined by use of our radionuclide method,¹ and cardiac output was determined by use of the radionuclide γ -function method (Fig. 1). The blood sample technique was used in the determination of blood volume.

The significance of the changes in the hemodynamic parameters before and after medication was tested by *t* test (paired comparison).

Results

The correlations between the changes in the intervillous blood flow and other hemodynamic parameters in different medication groups are shown in Table II. A marginally significant correlation was found between changes in intervillous blood flow and in diastolic blood pressure and a negative correlation between changes in intervillous blood flow and in blood volume in the furosemide group. The means and standard deviations of the hemodynamic parameters before and after medication are seen in Table III. In the control group the

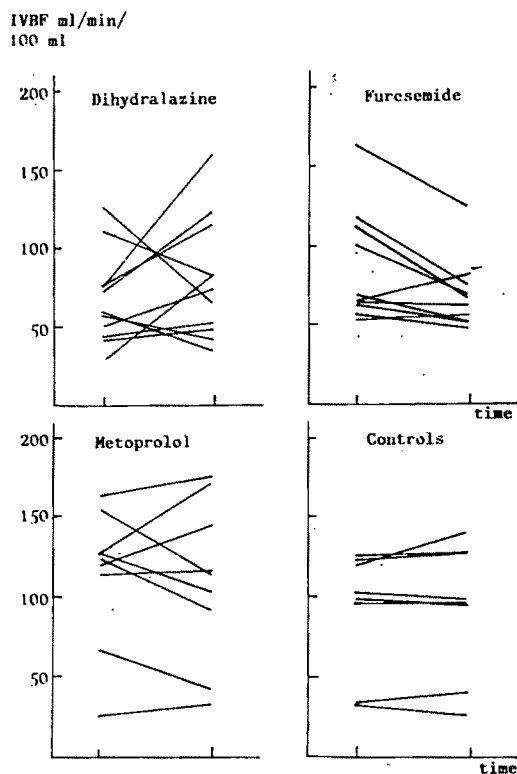


Fig. 2. The individual changes in the intervillous blood flow (IVBF) in different patient groups.

changes are insignificant. In the dihydralazine group the heart rate and cardiac output increased while the diastolic blood pressure fell. In the furosemide group the cardiac output, blood volume, and intervillous blood flow all showed a decrease. Metoprolol caused a decrease in heart rate, in both systolic and diastolic blood pressures, and in cardiac output.

The individual responses of the intervillous blood flow to different drugs are seen in Fig. 2.

Dihydralazine increased the intervillous blood flow in seven patients and decreased it in four patients. Furosemide decreased the intervillous blood flow in eight of 10 patients, whereas metoprolol caused an improvement in the intervillous blood flow in four patients and a reduction in four patients.

Two patients in the dihydralazine group were excluded from the series of tests because of a hypotensive reaction shortly after medication was given.

Comment

It is a commonly held viewpoint that intervillous blood flow can be improved by lowering the blood pressure in pregnancy-induced hypertension. In our study we were not able to find these types of correlation between the intervillous blood flow and other maternal hemodynamic parameters.

An intramuscular dose of 20 mg of furosemide ap-

peared to be ineffective as a hypotensive agent, showing a decrease in cardiac output, blood volume, and placental circulation. A negative effect on the intervillous blood flow took place in nearly all patients, and this confirms the common opinion that diuretics are contraindicated in pregnancy-induced hypertension.

A typical increase in heart rate and cardiac output was observed in our study 3 hours after the administration of dihydralazine mesylate, whereas the effect on the blood pressure was only mild. Ring et al.³ have demonstrated that intravenous hydralazine increases uterine blood flow in hypertensive pregnant sheep. Jouppila et al.⁴ and Lunell et al.⁵ have studied the effects of the intravenous administration of hydralazine 1 hour after its application in hypertensive pregnant women, and they were not able to show any changes in the mean intervillous blood flow. In spite of that, the intervillous blood flow was decreased in Lunell et al.'s study in nine of 13 patients compared with our four of 10 patients. Jouppila et al. did not give individual results in their paper.

Vink et al.⁶ have shown that intravenous injections of boluses of 6.25 as well as 12.5 mg dihydralazine result in signs of fetal distress in the presence of intra-uterine growth retardation. They concluded that intravenous bolus injections of dihydralazine were not recommended because of unpredictable falls in blood pressure unless the fetus could be delivered soon after the administration of the drug. Also in our small sample there were two mothers who suffered a hypotensive reaction, but in these cases after intramuscular administration. Thus dihydralazine seems to have negative effects independent of the method of administration and also without showing greater changes in blood pressure. When used, it should not be administered without simultaneous monitoring of maternal blood pressure and fetal heart rate.

Among the β -blocking hypotensive agents, metoprolol has been used in the treatment of pregnancy-induced hypertension both alone and in combination with other drugs.⁷ The results have been favorable when judged by criteria such as fetal intrauterine growth, Apgar score and perinatal mortality, and umbilical arterial Astrup values.⁸ However, our study shows that intervillous blood flow decreased in four to nine cases after the administration of methoprolol. Again, a great diversity of effect was observed, resembling the results of the dihydralazine study. That is why the treatment with metoprolol should be undertaken with the same precautions recommended in connection with dihydralazine therapy. We also know now that the determination of plasma levels of metoprolol in routine therapy is of limited value because of the poor correlation between therapeutic effect and plasma concentration of metoprolol in the individual patient.⁹

Naturally, it would have been ideal to limit our study to primigravid patients only, but the study would have taken an unreasonably long time because of lack of suitable volunteers. Anyway, no differences were noted between the primigravid and multigravid patients in each of the parameters measured.

Because of the great diversity of the effects of different drugs on maternal hemodynamics and the difficulties in the evaluation of fetal risks in different materials of pregnancy-induced hypertension, it is inadvisable to consider perinatal mortality in the estimation of the suitability of a hypotensive agent as a drug. In fact, it was not considered when diuretics were prohibited in the treatment of pregnancy-induced hypertension.

A new approach to a more careful policy in hypotensive treatment has been tried by restricting the hypotensive therapy to certain drugs in cases without fetal growth retardation. Naturally, this will rule out the most severe cases of pregnancy-induced hypertension in which hypotensive therapy is used due to maternal indications, which are the most important indications for hypotensive therapy.

An ideal hypotensive agent would be one that possesses a well-regulated hypotensive effect with a positive effect on intervillous blood flow, but this drug has yet to be invented. More detailed studies of the effects of older drugs on maternal hemodynamics during orthostasis in pregnancy-induced hypertension are still lacking. That is why conservatism in the use of hypo-

tensive drugs and a careful monitoring of the fetus when hypotensive therapy is started is the best policy available at present to prevent harm being done to the patient with intrauterine growth retardation.

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Plasma vasoactive intestinal polypeptide, insulin, gastric inhibitory polypeptide, and blood glucose in late pregnancy and during and after delivery

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The plasma levels of the gastrointestinal regulatory peptides vasoactive intestinal polypeptide, insulin, and gastric inhibitory polypeptide, as well as blood glucose, were measured in six healthy women before, during, and after normal parturition at term. Plasma levels of vasoactive intestinal polypeptide increased significantly ($p < 0.05$) during delivery and remained significantly elevated for 15 minutes post partum. Plasma insulin levels rose significantly ($p < 0.05$) within 5 minutes after delivery and stayed three-fold and significantly elevated for 120 minutes post partum. Plasma levels of gastric inhibitory polypeptide decreased significantly ($p < 0.05$) between 5 and 30 minutes after delivery. Blood glucose levels were significantly ($p < 0.05$) increased during labor, delivery, and the early postpartum period compared to late pregnancy. These findings suggest a mediating role for vasoactive intestinal polypeptide, insulin, and gastric inhibitory polypeptide in the physiologic adaptations to normal pregnancy, delivery, and the postpartum period. (AM J OBSTET GYNECOL 1986;155:126-31.)

Key words: Pregnancy, vasoactive intestinal polypeptide, insulin, gastric inhibitory polypeptide, blood glucose

Recently a number of gut-brain peptides, exhibiting a wide range of actions, have been characterized. Among these peptides, some (for example, motilin, somatostatin, pancreatic polypeptide, and vasoactive intestinal polypeptide) have both motoric and secretory effects on the gastrointestinal tract. In addition, vasoactive intestinal polypeptide has been shown to exert specific effects on the female genital tract, for example, relaxation of the uterine smooth muscle and increase of the myometrial, endometrial, and vaginal blood flow.¹ Insulin having metabolic effects is the major hormonal regulator of the blood glucose, which is the most important energy source during parturition. Gastric inhibitory polypeptide is another gut-brain peptide, whose main action probably is enhancement of insulin secretion. Thus these peptides may play a mediating role in the physiologic adaptations to normal pregnancy, delivery, and the postpartum period. Previously

we have reported on the changes of plasma motilin, somatostatin, and pancreatic polypeptide before, during, and after parturition.² In this study we report on changes in plasma vasoactive intestinal polypeptide, insulin, gastric inhibitory polypeptide, and blood glucose during late pregnancy, delivery, and post partum.

Material and methods

Patients. Six healthy women (mean age, 24.7 years; range, 17 to 35 years) participated in the study after informed consent was obtained. All women completed an uncomplicated gestation with normal vaginal delivery of healthy babies at term. Three women were primiparous and three multiparous. The patients were all hospitalized during early labor. They received no nutrients until 2 hours post partum. Analgesics were given to all women in the form of nitrous oxide/oxygen on a mask, and three also received pethidine, 75 mg intramuscularly 3 to 5 hours before delivery. On delivery, oxytocin, 10 IU intramuscularly, was routinely given to all six women.

Blood samples. Ethylenediaminetetraacetate-plasma was sampled as previously described³ and stored at -20°C until assayed. Three blood samples were drawn at 10-minute intervals at 2 to 4 cm cervical dilatation; at 6 to 8 cm cervical dilatation; at approximately 10, 5, and 0 minutes before delivery; and at 5, 15, 30, 60, and 120 minutes after delivery. The blood samples during labor were related to uterine contractions, for example,

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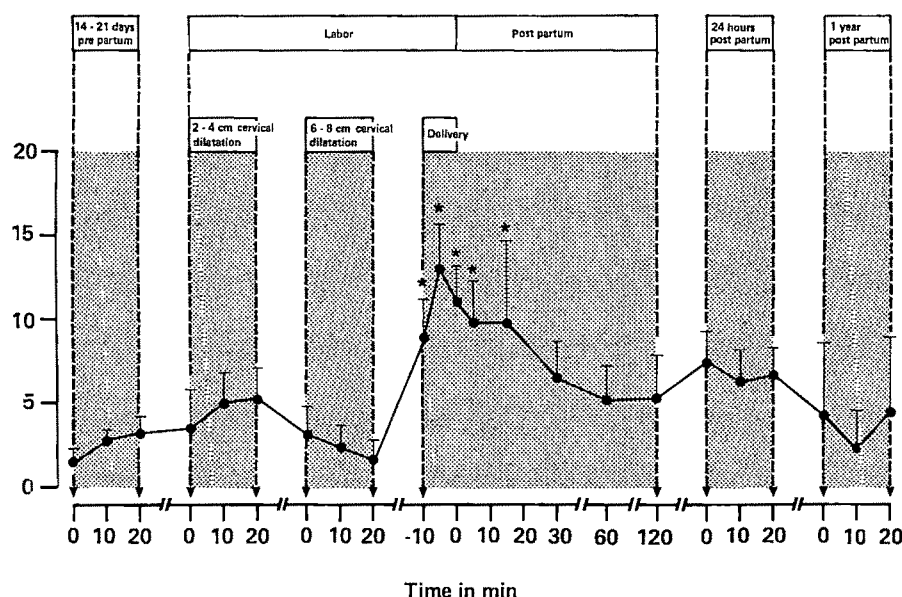


Fig. 1. Plasma vasoactive intestinal polypeptide (VIP) before, during and after parturition in six healthy women undergoing normal deliveries at term. Each point represents the mean value of six observations, and the vertical bars the standard errors of the means. The asterisks indicate significant difference from the values in late pregnancy ($p < 0.05$).

during and between contractions. In addition, three fasting blood samples were taken at 10-minute intervals 2 to 3 weeks before, 24 hours after, and 1 year after delivery.

Assays. Plasma levels of vasoactive intestinal polypeptide, insulin, and gastric inhibitory polypeptide were measured by radioimmunoassay methods as previously described.⁴⁻⁶ Blood glucose was measured by a hexokinase method (Gluco-quant, Boehringer, Mannheim, West Germany).

Statistical analyses. Statistical analyses were performed by a Wilcoxon paired rank sum test, and $p < 0.05$ was considered statistically significant.

Results

The results are presented in Figs. 1 to 4 and expressed as means $\pm$ standard errors of the means.

Vasoactive intestinal polypeptide. Plasma levels of vasoactive intestinal polypeptide remained almost constant until delivery. They rose significantly during delivery and remained significantly elevated for 15 minutes after parturition. Thereafter, plasma levels of vasoactive intestinal polypeptide decreased and remained unchanged at 24 hours and 1 year post partum (Fig. 1).

Insulin. No significant changes in plasma insulin were observed during labor and parturition. However, a significant rise in plasma insulin levels occurred within 5 minutes after delivery. Between 15 and 120 minutes post partum, an almost three-fold and significant increase in plasma insulin levels was observed. At 24

hours and 1 year after delivery, plasma insulin levels were similar to the prepartum values (Fig. 2).

Gastric inhibitory polypeptide. Plasma gastric inhibitory polypeptide remained unchanged during labor and parturition. Between 5 and 30 minutes post partum a significant decrease in plasma gastric inhibitory polypeptide concentrations was noted. Then, plasma gastric inhibitory polypeptide normalized, and no significant changes were found at 24 hours and 1 year post partum (Fig. 3).

Blood glucose. Blood glucose levels were significantly increased during labor and delivery as compared to the values in late pregnancy. A further significant increase was recorded in the first 30 minutes after delivery. Blood glucose levels 24 hours and 1 year post partum were still significantly elevated compared to blood glucose concentrations in late pregnancy, reflecting the lowered blood glucose levels in pregnancy (Fig. 4).

Relation to uterine contractions. No significant difference in plasma concentrations of vasoactive intestinal polypeptide, insulin, gastric inhibitory polypeptide, and blood glucose were observed during and between uterine contractions (Table I).

Comment

Previously Ottesen et al.⁷ have reported on plasma vasoactive intestinal polypeptide concentrations during pregnancy and labor. They found significantly higher plasma vasoactive intestinal polypeptide levels during the first and second stages of labor than during preg-

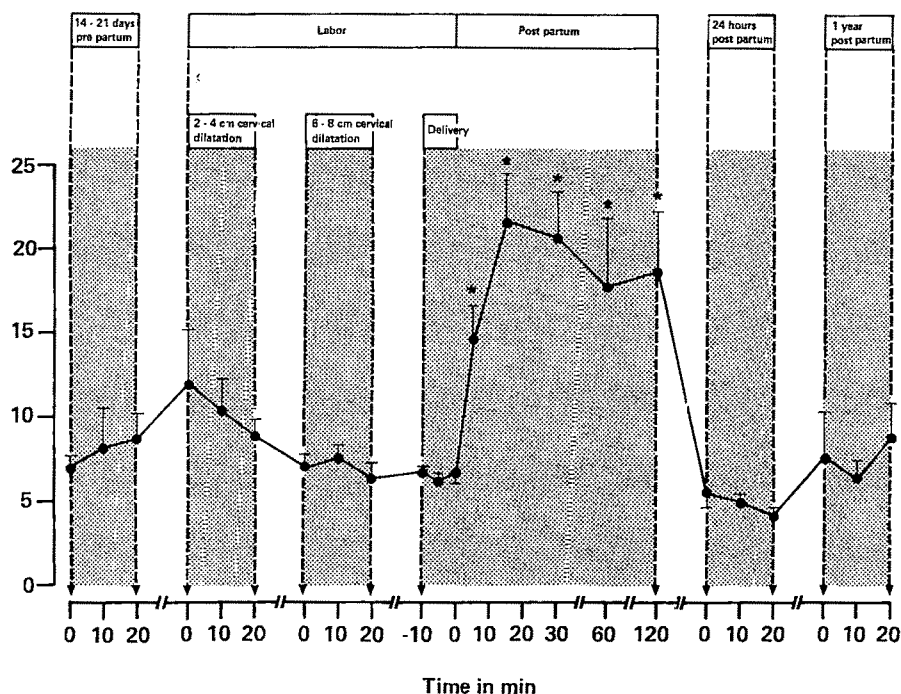


Fig. 2. Plasma insulin before, during, and after parturition in six healthy women undergoing normal deliveries at term. Each point represents the mean value of six observations, and the vertical bars represent the standard errors of the means. The asterisks indicate significant difference from the values in late pregnancy ($p < 0.05$).

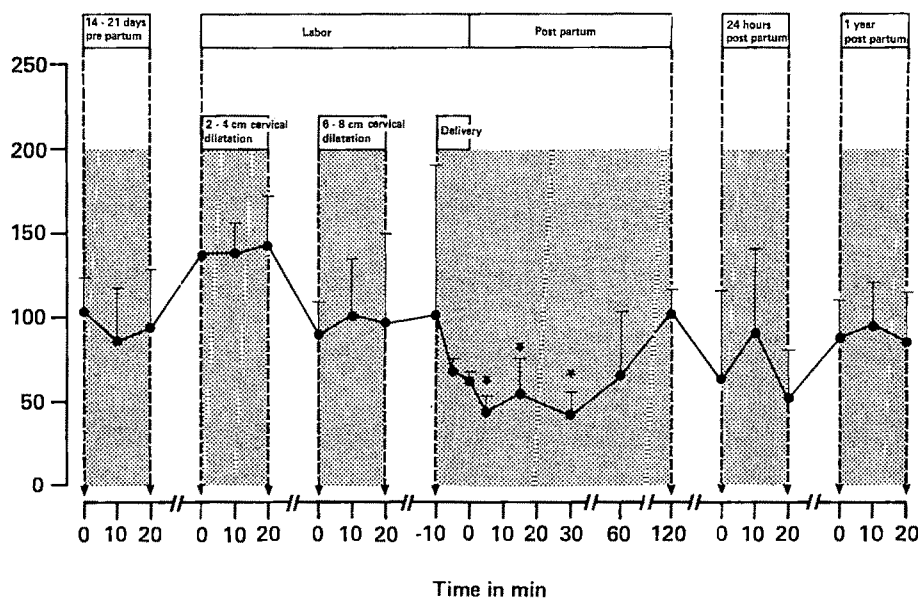


Fig. 3. Plasma gastric inhibitory polypeptide (GIP) before, during, and after parturition in six healthy women undergoing normal deliveries at term. Each point represents the mean value of six observations, and the vertical bars represent the standard errors of the means. The asterisks indicate significant difference from the values in late pregnancy ($p < 0.05$).

nancy. We found, on the other hand, only a significant rise in plasma vasoactive intestinal polypeptide levels in the second stage of labor and in the early postpartum period. These apparent discrepancies are hard to explain. Possible explanations for the increased plasma

vasoactive intestinal polypeptide levels during labor might be an increased local release from the genital tract or general increased release resulting from various hormonal factors or both.

Immunohistochemical studies have shown that va-

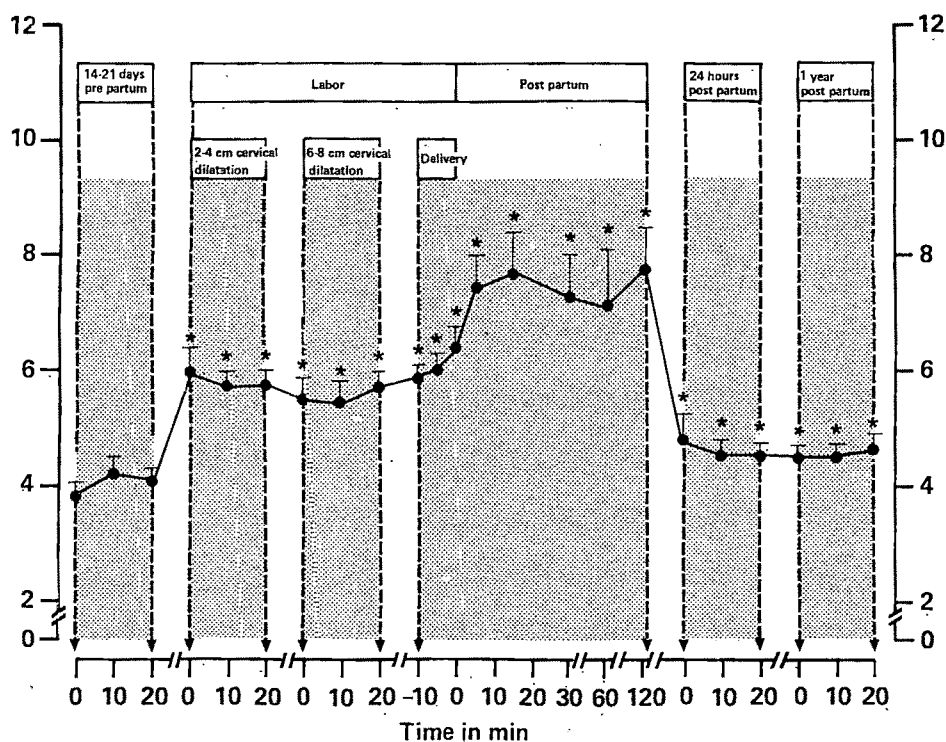


Fig. 4. Blood glucose before, during, and after parturition in six healthy women undergoing normal deliveries at term. Each point represents the mean value of six observations, and the vertical bars represent the standard errors of the means. The asterisks indicate significant difference from the values in late pregnancy ($p < 0.05$).

Table I. Plasma concentration of vasoactive intestinal polypeptide, insulin, gastric inhibitory polypeptide, and blood glucose (mean $\pm$ SEM) during labor, as related to uterine contractions

Period	Vasoactive intestinal polypeptide (pmol/L)	Insulin (μ U/ml)	Gastric inhibitory polypeptide (pmol/L)	Blood glucose (mmol/L)
First stage of labor (cervix, 2-4 cm)				
During contractions	5.6 $\pm$ 1.8	9.4 $\pm$ 1.7	145.0 $\pm$ 22.5	5.7 $\pm$ 0.3
Between contractions	3.5 $\pm$ 1.5	11.2 $\pm$ 2.1	124.0 $\pm$ 7.0	5.8 $\pm$ 0.3
First stage of labor (cervix, 6-8 cm)				
During contractions	3.0 $\pm$ 1.3	7.3 $\pm$ 0.8	86.0 $\pm$ 16.0	5.3 $\pm$ 0.4
Between contractions	1.5 $\pm$ 0.9	6.4 $\pm$ 0.6	86.5 $\pm$ 23.1	5.5 $\pm$ 0.3
Second stage of labor				
During contractions	15.7 $\pm$ 4.2	6.0 $\pm$ 0.5	61.0 $\pm$ 9.0	6.1 $\pm$ 0.3
Between contractions	11.8 $\pm$ 3.6	6.9 $\pm$ 0.2	90.7 $\pm$ 52.6	6.0 $\pm$ 0.3

soactive intestinal polypeptidergic nerve fibers are most abundant in the vagina and cervix.¹ Therefore mechanical pressure by the presenting part of the fetus on these tissues during delivery can probably cause an increased local release of vasoactive intestinal polypeptide. Alternatively, various hormonal changes may contribute to the elevated plasma vasoactive intestinal polypeptide concentrations during the second stage of labor. Thus oxytocin has been reported to increase portal as well as peripheral plasma vasoactive intestinal polypeptide levels in animals.⁸ Another possibility is that prostaglandins may evoke an increase in plasma vasoactive intestinal polypeptide during parturition, since interactions between prostaglandins and vasoactive in-

testinal polypeptide have been reported and the prostaglandins do indeed increase at delivery. It is also possible that opioids, which are known to increase during labor and delivery, can affect the vasoactive intestinal polypeptide level and particularly so since an effect on other gastrointestinal regulatory peptides has been reported.⁹ Finally, plasma vasoactive intestinal polypeptide levels have been reported to increase during exercise, and it has been proposed that vasoactive intestinal polypeptide can play a mediatory role in the metabolic, circulatory, and thermoregulatory adaptations to exercise.¹⁰ An exercise-induced increase in vasoactive intestinal polypeptide can therefore possibly also contribute to the observed elevation of plasma

vasoactive intestinal polypeptide levels during delivery.

These considerations cannot, however, be applied for the sustained vasoactive intestinal polypeptide levels during the early postpartum period, since the half-life of vasoactive intestinal polypeptide has been estimated to be 2 minutes. It is tempting to relate this rise to the autotransfused blood from the uterine blood pool after delivery, since this compartment probably contains relatively large quantities of vasoactive intestinal polypeptide. Another possibility is fetomaternal transfusion during delivery and expulsion of the placenta, since vasoactive intestinal polypeptide levels in cord plasma are substantially higher than in maternal plasma.⁷

No significant differences were found between plasma vasoactive intestinal polypeptide levels during and between uterine contractions. This confirms the findings of Ottesen et al.⁷

In accordance with our observations, previous authors have reported that plasma insulin does not change during labor. Hornnes¹¹ has found, however, higher fasting plasma insulin concentrations in pregnancy than post partum, whereas the immediate postpartum period was not studied in detail by his group. To our knowledge, we report here for the first time a substantial increase in plasma insulin concentration in the early postpartum period. The insulin increase could partly be due to the increased blood glucose levels also observed after delivery. However, no correlation was found between the blood glucose and plasma insulin levels. Besides blood glucose, insulin secretion is influenced by amino acids and various gastrointestinal regulatory peptides, for example, gastric inhibitory polypeptide, secretin, gastrin, and cholecystokinin. Of these peptides, neither gastric inhibitory polypeptide nor secretin showed any significant changes on delivery that could explain the rise in the plasma insulin levels (personal observation). On the contrary, plasma gastric inhibitory polypeptide levels decreased during the early postpartum period, thus possibly counteracting a further increase in plasma insulin concentrations. We have previously found a nonsignificant increase in plasma somatostatin during labor, delivery, and post partum.² Local gastrointestinal release of somatostatin in appreciable amounts usually induces only small changes in peripheral plasma concentrations, and somatostatin has been found to inhibit gastric inhibitory polypeptide release.¹² It is therefore tempting to speculate whether somatostatin may serve as a paracrine substance during labor, resulting in decreased plasma gastric inhibitory polypeptide levels. Another possibility is that the insulin increase could be mediated by oxytocin. As for vasoactive intestinal polypeptide, oxytocin has been shown to elicit insulin release in animals.¹³ Whether the same is true in humans as well is not known. If so, the intramuscular injection of oxytocin given to our patients at the time of delivery could explain the insulin rise.

However, plasma insulin remained unchanged during oxytocin infusion (10 μ U/kg/min) for the induction of labor (personal observation).

A striking similarity exists between the patterns of plasma insulin and plasma prolactin in the early postpartum period.¹⁴ The possibility exists therefore that these two hormones may be interdependent. A possible common mechanism for their release may be via β -endorphin release, since this substance stimulates prolactin as well as insulin secretion,^{15,16} and β -endorphin does indeed rise at delivery.

As previously reported,² blood glucose rose during labor and the early postpartum period. This is probably caused by the release of glucose-mobilizing agents such as cortisol and norepinephrine during maternal stress.

In conclusion, plasma vasoactive intestinal polypeptide levels rose significantly during delivery and remained significantly elevated for the initial 15 minutes post partum. This vasoactive intestinal polypeptide increase can most likely be ascribed to local mechanically induced release from the genital tract, to oxytocin, and to the effect of physical strain during labor. Plasma insulin concentration showed a three-fold and significant increase in the early postpartum period. The insulin increase can probably be related to the elevated blood glucose level, administration of oxytocin, and/or elevated opioid peptides at delivery. The physiologic significance of these observations are not clear, but vasoactive intestinal polypeptide may possibly play a beneficial role during delivery because of its muscle-relaxing and vasodilating effects. Similarly, the insulin rise post partum may be of importance for the metabolic adaptations taking place after delivery.

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Fetal acoustic stimulation testing

II. A randomized clinical comparison with the nonstress test

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Antepartum fetal heart rate testing, specifically the nonstress test, is of accepted value in the antenatal surveillance of high-risk pregnancies. Fetal rest-activity cycles coupled with arbitrary test intervals appear to lead to falsely nonreactive tests. Methods to alter fetal behavioral states have not been uniformly successful. A retrospective analysis of the adjunctive use of acoustic stimulation at our institution demonstrated a 50% reduction in the number of nonreactive tests. Consequently a prospective randomized clinical trial was undertaken to compare the standard nonstress test with the fetal acoustic stimulation test. Those patients randomized to the fetal acoustic stimulation test underwent transabdominal acoustic stimulation with a Model 5C electronic artificial larynx. The incidence of nonreactive tests was 14% in the control group and 9% in the study group ($\chi^2 = 11.09$, $p = 0.004$). A significant reduction in testing time was also observed. The fetal acoustic stimulation test offers advantages over the traditional nonstress test by lowering the incidence of nonreactive tests and reducing testing time. (*AM J OBSTET GYNECOL* 1986;155:131-4.)

Key words: Antepartum fetal heart rate testing, fetal acoustic stimulation

Antepartum fetal heart rate testing is of accepted value in the antenatal surveillance of high-risk pregnancies. In particular, the nonstress test has been shown to be a safe and reliable means of assessing fetal well-being.¹⁻³ Less well defined is the ability of the nonstress test to predict fetal compromise. This is evidenced by the relatively low incidence of positive contraction stress tests after nonreactive nonstress tests^{1-4,5} In an effort to reduce the incidence of falsely nonreactive tests, presumably secondary to fetal sleep states, investigators

have used mechanical stimulation, glucose administration, and exposure of the fetus to light and sound, all with disappointing results.⁶⁻⁹ We recently reported the apparent effectiveness of transabdominal acoustic stimulation of the fetus with a Model 5C electronic artificial larynx (Western Electric, New York) in reducing by 50% the incidence of nonreactive nonstress tests.¹⁰

Consequently a prospective randomized clinical trial was undertaken to compare the FAS-TEST with the nonstress test.

Material and methods

After giving informed consent, patients presenting to the Antepartum Fetal Testing Unit of Women's Hospital, Los Angeles County/University of Southern California Medical Center were randomized by lottery to receive either a nonstress test (group I) or the FAS-TEST (group II). No patients were excluded from this

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Table I. Indications for antepartum fetal heart rate testing

	FAS-TEST		Nonstress test		Refused	
	No.	%	No.	%	No.	%
Postdates	145	39.6*	162	46*	50	27.5
Intrauterine growth retardation	40	10.9	38	10.9	37	20.3
Diabetes	44	12.0	39	11.2	23	12.6
Decreased fetal movement	63	17.2	49	14.0	34	18.7
Prior stillbirth	13	3.6	5	1.4	4	2.2
Other	61	16.7	56	16.1	34	18.7
Total	366	100	349	100	182	100

* $\chi^2 = 3.38$, $p < 0.05$.**Table II.** The incidence of reactive tests

	Reactive		Non-reactive		Incomplete	
	No.	%	No.	%	No.	%
FAS-TEST	773	90*	78	9	7	1
Nonstress test	742	85*	122	14	5	1
Refused	431	86	67	13	5	1

* $\chi^2 = 11.09$, $p = 0.004$.

investigation. Once assigned, these patients remained in these groups until delivery. Patients who declined to participate in the study (group III) or those randomized to the nonstress test group underwent antepartum fetal heart rate testing by existing protocol. Patients randomized to the FAS-TEST group underwent monitoring for a period of 5 minutes. If criteria for reactivity were not met, transabdominal acoustic stimulation overlying the fetal vertex was accomplished with an electronic artificial larynx. Sound pressure levels of this device measured at 1 m in air averaged 82 db. Spectral analysis of the electronic artificial larynx revealed a fundamental frequency of approximately 80 Hz, and harmonics ranging from 20 to 9000 Hz were also present. The fetus was stimulated for ≤ 3 seconds. If no qualifying acceleration was noted, the stimulus was repeated at 1-minute intervals for a maximum of three times.

All tests were performed in the Antepartum Fetal Testing Unit by specially trained nurses with the patient in semi-Fowler's position. Blood pressure determinations were made every 5 minutes and recorded. Tests were considered reactive if there were two or more fetal heart rate accelerations of at least 15 bpm, lasting 15 seconds, in a 10-minute period. Nonreactive tests, defined as 40 minutes without two qualifying accelerations, were followed by a repeat nonstress test that same day, a biophysical profile, or a contraction stress test depending on the clinical situation.¹¹

Table III. Mean duration of fetal heart rate test

Test	Mean duration (min)	Significance
FAS-TEST	22.6 $\pm$ 15.67	—
Reactive	20.34 $\pm$ 14.0	—
Nonreactive	45.19 $\pm$ 13.36	NS
Nonstress test	27.07 $\pm$ 16.67	$p < 0.001^*$
Reactive	24.38 $\pm$ 15.15	$p < 0.001^\dagger$
Nonreactive	45.07 $\pm$ 12.68	NS
Refused	27.49 $\pm$ 15.96	$p < 0.001^\ddagger$
Reactive	24.4 $\pm$ 15.15	$p < 0.001^\S$
Nonreactive	45.7 $\pm$ 12.13	NS

* $t = 5.73$. $^\dagger t = 5.38$. $^\ddagger t = 5.33$. $^\S t = 4.88$.

In all three groups the following data were analyzed: indications for testing, parity, percentage of reactive tests, test length, and the antenatal fetal death rate within 7 days of a reactive test.

Results

A total of 897 patients underwent a total of 2230 antepartum fetal tests; 366 patients were randomized to the FAS-TEST and 349 to the nonstress test. An additional 182 patients refused participation in this investigation and served as a second control group.

Table I lists the five most common indications for antepartum fetal heart rate testing within each group. The mean parity of each group was statistically similar. The duration of pregnancy ranged from 28 to 44 weeks with the majority being term (37 to 41 weeks). The only difference noted was a significantly greater number of postdates patients in the nonstress test group.

A total of 210 tests (25%) in the FAS-TEST group were reactive within the first 5 minutes and did not require stimulation. A reduced frequency of nonreactive tests was observed in the FAS-TEST group (Table II). This was statistically different from either control group. To analyze the effect of the larger number of postdates patients in the nonstress test group, the percentage of nonreactive tests in only the postdates patients was determined. No difference could be identified. Also reduced was the mean duration of reactive tests in the study as compared with either control group (Table III).

Table IV demonstrates the distribution of reactive FAS-TEST and the number of stimuli used in relation to the gestational age of the fetus. Analysis of variance was performed on these data and demonstrated a significant reduction in the number of required stimuli with advancing gestational age ($F = 3.05$, $p = 0.029$).

One antenatal death occurred within 7 days of a reactive test. This patient, in the nonstress test group, underwent antepartum fetal heart rate testing for post-

Table IV. Number of required stimuli

Number of stimuli	Weeks of gestation							
	27-32 (n = 54)		33-36 (n = 183)		37-41 (n = 231)		≥42 (n = 137)	
	No.	%	No.	%	No.	%	No.	%
1	33	61	118	65	169	73	104	76*
2	8	15	40	22	35	15	20	15*
3	13	24	25	13	27	12	13	9*

*Analysis of variance, $F = 3.03$, $p = 0.029$.

dates. Fetal death occurred 2 days following a reactive nonstress test. No apparent cause for the death was noted.

Comment

A reactive nonstress test is highly predictive of the absence of fetal death within 7 days.^{3, 12} Alternatively, a nonreactive nonstress test, although associated with a higher perinatal mortality, is infrequently followed by a positive contraction stress test and subsequent fetal death. If the efficiency of antepartum fetal heart rate testing is to be improved, a reduction in the frequency of nonreactive nonstress testing is desirable.

As early as 1925, Peiper¹³ reported fetal movement in response to acoustic stimulation. This association was later confirmed by others.^{14, 15} In addition, fetal heart rate changes were also noted after acoustic stimulation.^{15, 16} Finally, these induced fetal heart rate accelerations were shown to be predictive of fetal well-being.^{9, 17, 18} However, an unacceptably high nonreactive, or rather nonresponse, rate was observed.

Birnholtz and Benacerraf¹⁹ reported a 100% blink-startle response to a vibroacoustic stimulus, produced by the electronic artificial larynx, in those fetuses of at least 28 weeks of gestation. Although their primary goal was detection of fetal deafness, the presence of a blink-startle response suggested fetal well-being.

We retrospectively analyzed our experience with adjunctive acoustic stimulation during antepartum fetal heart rate testing and found a 50% reduction in the number of nonreactive tests.¹⁰ This formed the basis for our prospective comparison. It is of interest to note the incidence of nonreactive tests was lower in both the standard nonstress test (12.6%) and the FAS-TEST (6.1%) groups in the retrospective investigation as compared to the current one. There was no apparent difference in the tested population or in the indications for fetal heart rate testing.

As noted in Table I, the three groups were comparable except for a higher percentage of postdates patients in the nonstress test group. Overall, we observed a reduction in nonreactive tests, from 14% in the nonstress test and 13% in the refused groups to 9% in the FAS-TEST group. To analyze the effect of the larger

number of postdates patients in the nonstress test group, the percentage of nonreactive tests in only the postdates patients was determined. No statistically significant difference in the percentage of reactive tests could be found. Therefore, the overall reduction in the number of nonreactive tests does not appear to be a result of the higher incidence of postdates patients in the control group.

In addition, the mean test length in the FAS-TEST study population was approximately 4½ minutes less than in either nonstress test. This same relationship exists even when one considers only reactive tests. The shortened test time is a product of reduced time to achieve a reactive test and fewer nonreactive tests. In a busy antepartum testing unit doing 50 tests per day, these 4 minutes per test result in a savings of 3¾ hours per day. Further time savings may be possible with earlier application of the stimulus.

Outcome, as measured by the antenatal fetal death rate, was no different by test type. However, this rare event will require much larger study groups to evaluate this particular outcome measure.

The reliability of acoustic stimulation as a predictor of fetal well-being is also supported by Read and Miller⁹ and Serafini et al.^{9, 17} Read and Miller compared the presence of an acceleration in response to a 5-second stimulus of 2000 Hz at 105 to 120 db with a contraction stress test. When an acceleration was present, a negative contraction stress test followed in 43 of 43 tests. In the absence of an acceleration, 5 of 8 (62.5%) fetuses demonstrated a positive contraction stress test. Serafini et al. showed no difference between spontaneously reactive fetuses and those who became reactive after a 1-second, 1220 Hz, 126 db stimulus in terms of antenatal fetal death, intrapartum distress, incidence of low 1- and 5-minute Apgar scores or the incidence of meconium-stained amniotic fluid.

The greater response rate noted in our investigation is most probably related to the type of stimulus chosen. Other authors have used primarily pure-tone stimuli of relatively high frequency (1000 to 2000 Hz). At this frequency the mechanical vibratory component is minimized. Our stimulus, at a fundamental frequency of 80 Hz, is perhaps more aptly described as vibroacoustic.

Trudinger and Boylan¹⁶ found that the percentage of fetuses which responded increased with the use of lower frequency acoustic stimuli.

In addition, alteration in the behavioral state of the fetus has been suggested to occur following acoustic stimulation.^{9, 20, 21} This is supported by the apparent consistent occurrence of fetal movement following stimulation with the electronic artificial larynx. Second, altered baseline fetal heart rate and variability were commonplace during the FAS-TEST. These changes have been suggested by some authors to be a reflection of fetal state changes.^{21, 22} Further investigation as to the effect of the FAS-TEST on fetal behavioral states is ongoing.

A final consideration is one of safety. Other authors using similar sound levels have found no adverse effects to its use.^{9, 17, 18} This is not surprising in view of high baseline levels of sound reported by earlier investigators.²³ In view of these findings, it is highly unlikely the levels and frequency of the sound used in this investigation are harmful.

In summary, the FAS-TEST offers distinct advantages to the traditional nonstress test. Fewer nonreactive tests (1) reduce patient and provider anxiety, (2) shorten overall testing time, and (3) allow perinatal resources to be more efficiently used. These advantages are even more noteworthy, since the predictive reliability of the FAS-TEST is comparable to the nonstress test. Investigation of the predictive reliability of the FAS-TEST as it relates to fetal death, fetal distress, meconium-stained amniotic fluid, and newborn outcome is currently underway. Although definitive advantages to the FAS-TEST exist, its routine implementation should await further investigation of its predictive reliability.

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Maternal dietary substrates and human fetal biophysical activity

I. The effects of tryptophan and glucose on fetal breathing movements

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To study the effects of L-tryptophan and glucose on fetal breathing activity, we examined 40 women with normal term pregnancies, randomly assigned to four equal groups who either continued fasting (group C), received 1 gm of oral tryptophan (group T), received 100 gm of oral glucose (group G), or received both substrates (group T + G). Studies lasted 210 minutes, during which fetal breathing movements were observed with real-time ultrasonography and entered and analyzed for incidence, rate, and variability on a microcomputer. Plasma glucose and tryptophan levels were determined every 30 minutes. The incidence of fetal breathing movements declined in group C and rose significantly in the other groups. Breathing rates were unchanged in groups C and T but rose significantly in groups G and T + G during peak breathing intervals. Breath interval variability did not change significantly in any study group. Maternal administration of tryptophan is associated with an alteration in fetal breathing activity but to a lesser degree than that observed after maternal glucose loading. (AM J OBSTET GYNECOL 1986;155:135-9.)

Key words: Tryptophan, glucose, fetal breathing movements

Fetal breathing movements are biophysical activities essential for both neuromuscular and biochemical maturation of the respiratory system. While their morphologic features and incidence are influenced by many environmental variables, their relationship to maternal dietary state has only been recently appreciated.¹ Oral or intravenous administration of glucose to third-trimester gravid women results in consistent increases in incidence of fetal breathing movements²⁻⁵ but not in breath rate or breath interval variability.⁶ However, the impact of other dietary substrates on fetal breathing movements has received little attention. Fetal respiratory activity is also related to behavioral state, which in turn may be affected by relative levels of brain-active amines, such as serotonin.⁸ Serotonin precursors, such as the amino acid L-tryptophan, appear to alter sleep patterns in normal term neonates.⁹ Recently, Quilligan et al.¹⁰ demonstrated that 5-hydroxytryptophan administration to fetal lambs increased both incidence and amplitude of fetal breathing movements, even during episodes of high-voltage electrocortical activity. As tryptophan is ubiquitous in normal prenatal diets, it would be important to examine its effects on the respiratory activity of normal term human fetuses.

The purpose of this study was to determine the breathing responses of human fetuses to maternal administration of a 1 gm oral tryptophan load, to compare them with those observed during fasting states or after a 100 gm glucose load, and to relate them to maternal plasma glucose and tryptophan concentrations.

Material and methods

Forty normal pregnant women, between 37 and 41 weeks' gestational age, were enrolled after giving informed consent. All patients had normal carbohydrate tolerance as determined by a 50 gm oral glucose loading test performed in the third trimester. Gestational age was determined by a combination of menstrual dates and ultrasound measurements of the fetal biparietal diameter, abdominal circumference, and femur length. No anomalies were present and all fetuses were considered appropriate for gestational age.

Before the studies, patients were on unrestricted diets, containing at least 200 gm of carbohydrate per day, and were receiving no medications other than prenatal vitamins. All patients fasted from midnight of the day preceding studies and abstained from smoking for at least 10 hours before each session. At 8:30 AM of the study days, patients were randomly assigned to one of four groups, each containing 10 subjects: (1) fasting

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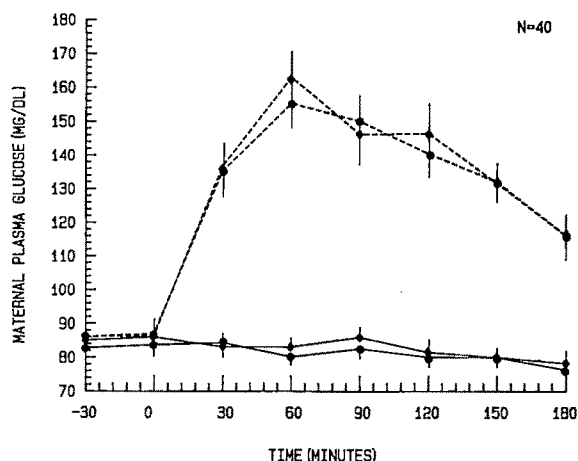


Fig. 1. Mean maternal plasma glucose concentrations at 30-minute intervals ($\pm$ SEM). ●—●, Fasting control patients; ◆—◆, patients receiving only tryptophan; ●...●, patients receiving only glucose; ◆---◆, patients receiving both tryptophan and glucose.

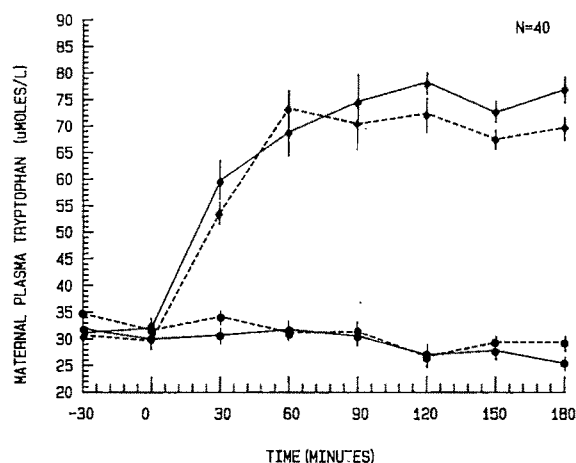


Fig. 2. Mean maternal plasma tryptophan concentrations at 30-minute intervals ($\pm$ SEM). ●—●, Fasting control patients; ◆—◆, patients receiving only tryptophan; ●...●, patients receiving only glucose; ◆---◆, patients receiving both tryptophan and glucose.

controls (group C), (2) those receiving oral tryptophan (group T); (3) those receiving oral glucose (group G), and (4) those receiving both tryptophan and glucose (group T + G).

A heparin-lock intravenous catheter was inserted at the beginning of each study. Maternal blood, in 8 ml samples, was drawn every 30 minutes during the study and stored at 4° C for subsequent determinations of plasma glucose and tryptophan levels. Plasma glucose levels were determined on an AutoAnalyzer, with the use of an enzyme reaction of hexokinase coupled with glucose-6-phosphate dehydrogenase. Plasma tryptophan levels were assayed by high-performance liquid chromatography with precolumn derivatization with the use of o-phthalaldehyde.

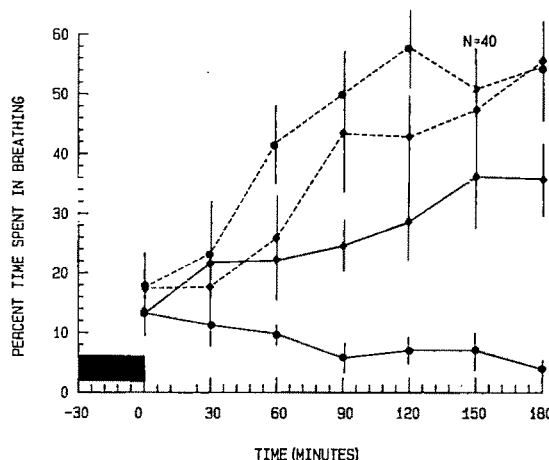


Fig. 3. Mean 30-minute incidences of fetal breathing movements ($\pm$ SEM) in fasting control patients (●—●), patients receiving only tryptophan (◆—◆), patients receiving only glucose (●...●), and patient receiving both tryptophan and glucose (◆---◆). The initial 30 minutes (solid bar) represents a fasting control period in all groups, following which glucose and/or tryptophan were given.

Observations of fetal breathing movements began at 9:00 AM ($t - 30$ minutes) and consisted of a 30-minute control period, followed by a 180-minute experimental period. At $t - 0$ minutes, the patients in group T received 1 gm of oral tryptophan; those in group G, 100 gm of oral glucose; those in group T + G, both tryptophan and glucose. Patients in group C continued fasting. All patients were permitted to drink tap water as desired during the studies. Fetal breathing activity was observed with a real-time ultrasound linear-array scanner, operating at 3.5 MHz (ADR 4000/L, ADR Corporation, Tempe, Arizona). A sagittal view of the fetal chest and abdomen was obtained and fetal breathing movements, defined as the paradoxical movement of chest and abdomen, were entered by trained observers, using a remote event indicator on a specially programmed microcomputer (IBM XT, 384 KRAM, IBM Corporation, Boca Raton, Florida). While the observers were not blinded as to the individual patient's treatment groups, the order of their observations was randomly rotated for each study. Fetal trunk movements were defined as fetal body displacement sufficient to prevent recognition of fetal breathing movements. Apnea was defined as the absence of fetal breathing movements and fetal trunk movements for more than 6 seconds.¹ Fail time was defined as periods during which neither fetal breathing movements nor fetal trunk movements could be entered because of technical problems or patient interruptions. Fail time averaged 2.7 ± 0.8 (SEM) for all groups and did not differ significantly among the groups.

During each study, fetal breathing movements were analyzed in successive 5-, 15-, and 30-minute intervals. Respiratory rate and breath interval variability (the co-

Table I. Distribution of the number and percent of 15-minute apneic intervals

Group	Total No. of intervals	<i>t</i> - 30 to <i>t</i> - 0		<i>t</i> - 0 to <i>t</i> + 60		<i>t</i> + 60 to <i>t</i> + 180	
		No.	%	No.	%	No.	%
C	140	4	20.0	3	7.5	31	38.7
T	140	4	20.0	5	12.5	4	5.0
G	140	3	15.0	5	12.5	5	6.3
T + G	140	5	25.0	5	12.5	5	6.3

efficient of variation of the mean breath interval) were also calculated during these same intervals when fetal breathing movements were present. Data were analyzed offline on a microcomputer, with a previously validated software program,¹¹ and were compared by *t* tests, analysis of variance with the use of repeated measures, or Fisher's exact test, where appropriate. Unless otherwise indicated, results are listed as means $\pm$ standard errors.

Results

Obstetric outcomes. Data are presented as mean $\pm$ SD. All groups were similar for gestational age (weeks) at the time of study (group C, 38.1 ± 1.2 ; group T, 37.7 ± 1.2 ; group G, 37.6 ± 1.4 ; group T + G, 37.6 ± 1.2), test-to-delivery intervals (days) (group C, 12.4 ± 11.3 ; group T, 12.9 ± 6.7 ; group G, 15.6 ± 9.4 ; group T + G, 13.3 ± 8.7), birth weight (grams) (group C, 3466 ± 302 ; group T, 3627 ± 339 ; group G, 3460 ± 492 ; group T + G, 3375 ± 462), and Apgar scores. There were 21 male and 19 female infants delivered, again distributed similarly among all groups. All infants exceeded the tenth percentile for gestational age, while one infant in each group exceeded the ninetieth percentile for gestational age. No intrapartum or neonatal complications occurred for study fetuses.

Plasma glucose (Fig. 1). The mean plasma glucose level, determined at 30-minute intervals, was similar for all groups at *t* - 30 minutes: group C, 82.7 ± 3.1 mg/dl; group G, 86 ± 2.9 mg/dl; group T, 85 ± 2.5 mg/dl; group T + G, 86 ± 3.4 mg/dl. Plasma glucose levels remained stable and similar in groups C and T throughout the study sessions. The plasma glucose level rose significantly ($p < 0.01$) and similarly in groups G and T + G at *t* + 30 minutes, peaked at *t* + 60 minutes, and remained elevated over control values for the remaining 120 minutes.

Plasma tryptophan (Fig. 2). In all groups, mean plasma tryptophan levels were similar at *t* - 30 minutes: group C, 32 ± 2.8 mol/L; group T, 31.2 ± 2.2 mol/L; group G, 34.7 ± 3.4 mol/L; group T + G, 30.7 ± 2.9 mol/L. Plasma tryptophan levels were stable and similar throughout the study sessions in groups C and G. The plasma tryptophan level rose significantly and similarly in groups T and T + G ($p < 0.01$) at *t* + 30 minutes, peaked at *t* + 60 minutes, and re-

mained significantly higher than control values for the remaining 120 minutes.

Fetal breathing movement incidence (Fig. 3). The 30-minute incidences of fetal breathing movements in all groups were statistically similar during the control period, averaging 15.4%. In group C, the incidence of fetal breathing movements declined significantly with time ($p < 0.05$, repeated measures of variance), while in the other groups, the incidence rose significantly with time (group T, $p < 0.05$; group G, $p < 0.001$; group T + G, $p < 0.015$; repeated measures of variance). Intergroup comparisons (paired *t* test, two-tailed) showed significant differences in incidences of fetal breathing movements between group C and the other three groups, beginning at *t* + 60 minutes ($p < 0.05$), which were sustained at all subsequent 30-minute intervals. Significant differences in incidence of fetal breathing movements between groups T and G were noted at *t* + 90 minutes ($p < 0.02$) and were sustained until *t* + 150 minutes. There were no significant differences in incidence of fetal breathing movements for any 30-minute interval between groups T and T + G or between groups G and T + G.

Peak incidence of fetal breathing movements occurred in group G (57.7%) at *t* + 120 minutes (60 minutes after peak maternal glucose levels), in group T + G (55.3%) at *t* + 180 minutes (120 minutes after peak maternal glucose and tryptophan levels), and in group T (36.1%) at *t* + 150 minutes (90 minutes after peak maternal tryptophan levels).

Table I summarizes the distribution of 15-minute intervals during which fetal breathing activity was absent. All groups were similar during the control period and first hour; there were significantly more fetuses with one or more 15-minute apneic intervals ($p = 0.0039$, Fisher's test) in the control group during the final 2 hours of study. There were no significant differences in the distribution of apneic intervals among groups T, G, and T + G. The longest apneic episode recorded during any study session lasted 60 minutes (group C).

Fetal breathing rates (Fig. 4). Mean 30-minute fetal breathing rates remained constant and similar in groups C (43.7 ± 2.0 breaths per minute) and T (40.2 ± 1.0 breaths per minute) throughout the studies. Mean 30-minute fetal breathing rates were signif-

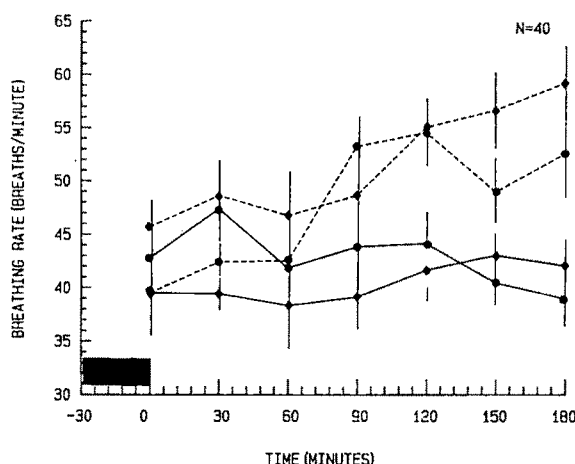


Fig. 4. Mean 30-minute fetal breathing rates ($\pm$ SEM) in fasting control patients ($\bullet$ — $\bullet$), patients receiving only tryptophan ($\blacklozenge$ — $\blacklozenge$), patients receiving only glucose ($\circ$ — $\circ$), and patient receiving both tryptophan and glucose ($\diamond$ — $\diamond$). The initial 30 minutes (solid bar) represents a fasting control period in all groups, after which glucose and/or tryptophan were given.

icantly higher ($p < 0.05$) for the total studies of groups G (51.9 ± 3.8 breaths per minute) and T + G (55.1 ± 2.4 breaths per minute) when compared with groups C and T. Intergroup comparisons of mean 30-minute fetal breathing rates (paired t test, two-tailed) showed significant differences ($p < 0.05$) at $t + 90$ minutes for group G when contrasted with either group C or group T that were sustained for all subsequent 30-minute intervals. Mean 30-minute breathing rates were significantly higher for group T + G, when compared with either group C or group T ($p < 0.01$) for all 30-minute intervals after $t + 120$ minutes. There were no significant differences for mean 30-minute breathing rates for any interval between groups G and T + G.

Breath interval variability. No significant trends were noted for any of the groups in the coefficients of variation of mean breath intervals, when analyzed in 5-minute, 15-minute (Fig. 5), or 30-minute intervals.

Comment

This study demonstrates that tryptophan, as well as glucose, when administered to normal term pregnant women, is associated with alterations in the pattern of fetal breathing activity. The changes in incidence of fetal breathing movements in the two glucose-treated groups are comparable to those of previous studies,²⁻⁵ although these latter involved different glucose loads, routes of administration, or gestational ages. The increase in fetal breathing rates after glucose loading is similar to that reported by Bocking et al.,⁸ who administered 25 gm of glucose intravenously to their subjects at term. The distribution of 15-minute apneic episodes and the onset and magnitude of peak breathing activity

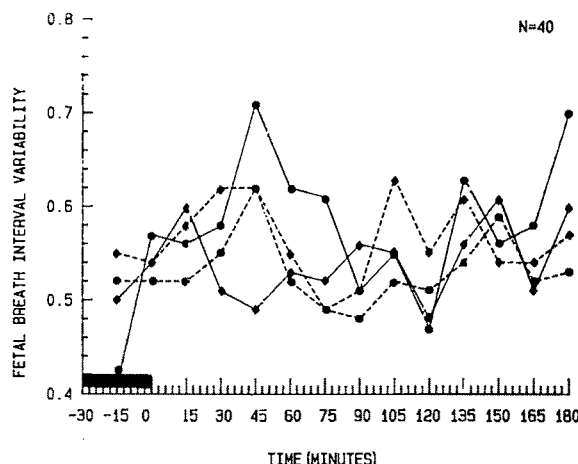


Fig. 5. Mean coefficients of variation of mean 15-minute breath intervals in fasting control patients ($\bullet$ — $\bullet$), patients receiving only tryptophan ($\blacklozenge$ — $\blacklozenge$), patients receiving only glucose ($\circ$ — $\circ$), and patient receiving both tryptophan and glucose ($\diamond$ — $\diamond$). The initial 30 minutes (solid bar) represents a fasting control period in all groups, after which glucose and/or tryptophan were given.

also agree with the observations of Natale et al.,² who used a 50 gm glucose load. While the exact means by which glucose stimulates fetal breathing activity is not certain, Richardson et al.¹² suggest that it may produce its effect through alterations in the incidence of low-voltage electrocortical activity, rather than by changes in cerebrospinal fluid acid-base balance.

The role of tryptophan in central respiratory regulation has been reviewed in adult and fetal animals.^{8, 13} In summary, tryptophan, acting as a substrate for increased synthesis of serotonin, may lead to stimulation of the central serotonergic neurons of the median raphe. Its access to the brain depends on the availability of blood-brain barrier amino acid carriers and the relative concentrations of other neutral amino acids¹⁴; its uptake may be enhanced by concurrent administration of carbohydrates.¹⁵ In pregnancy, fetal amino acid availability is partly determined by placental active transport systems; unfortunately there is little information on the actual kinetics of these systems in human gestation.¹⁷

Observations of the relationship of tryptophan and its metabolites to fetal breathing activity have been limited to those of Quilligan et al.¹⁰ and are in conflict with those reported in adult animals by Armijo and Florez.¹⁶ While the former group demonstrated an increase in incidence of fetal breathing movements after direct infusion of 5-hydroxytryptophan to preterm fetal lambs, the latter found that 5-hydroxytryptophan caused respiratory depression in conscious adult cats. The present study is an initial effort to gain information on the effects of tryptophan on human intrauterine respiratory. Our data suggest that fetal breathing activity, as reflected by a progressive increase in incidence of fetal

breathing movements and a reduction in the frequency of apneic episodes, is altered after maternal administration of tryptophan. In contrast with the fetal breathing patterns observed after maternal administration of glucose, maternal administration of tryptophan is associated with lower peak increases in incidence of fetal breathing movements that occur later in time and are not accompanied by altered respiratory rates. As plasma glucose levels of tryptophan-treated patients did not change, it is unlikely that these fetal respiratory effects are produced by a mechanism similar to that produced by glucose administration. Moreover, it appears that the concurrent administration of glucose and tryptophan results in a substantial delay in peak breathing incidence. This suggests that the interaction of maternal substrates may be important and should be considered in the interpretation of fetal breathing responses to complex, multicomponent maternal diets.

As human fetal breathing activity becomes more extensively studied, it appears that its regulation may involve numerous biochemical pathways. It is probable that the relationship between brain-active amines or their precursors and central respiratory activity is quite complex and subject to both maturational and dose-dependency effects. The observations of tryptophan-induced changes in sleep behavior of normal term neonates, reported by Yogman and Zeisel,⁹ support the hypothesis that central serotonergic receptors may be operative in behavioral modulation of term fetuses. Studies of tryptophan dose-response and placental transport kinetics in human pregnancy are needed to improve the understanding of these phenomena.

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Protein S deficiency in pregnancy

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The case of a primigravid patient with protein S deficiency, a rare disorder resulting in recurrent venous thrombosis, was managed with prophylactic heparin therapy and elective pregnancy termination. Although rarely diagnosed, protein S deficiency may become more commonly identified with wider application of protein S assays. (AM J OBSTET GYNECOL 1986;155:140-1.)

Key words: Protein S deficiency, pregnancy, venous thrombosis

Pregnancy is a high-risk period for the development of thromboembolic disease, and pulmonary embolism is the third leading cause of maternal death in the United States. A variety of factors, including venous stasis, tissue thromboplastin release, and altered coagulation levels may contribute to this increased risk. Hereditary deficiency of antithrombin III is associated with recurrent thromboembolic disease, and the risk of these complications is increased during pregnancy. Protein S deficiency, another hereditary thromboembolic disorder resulting in recurrent venous thrombosis, was first reported in 1984 in 12 patients.^{1,2} We report a primigravid patient with recurrent venous thrombosis and documented protein S deficiency. Pregnancy complicating protein S deficiency has not been reported previously, but may soon be recognized more frequently as testing becomes more widely available and our understanding of recurrent venous thrombosis increases.

Case report

A 15-year-old, primigravid, unmarried, white girl with an unplanned pregnancy of 17 weeks gestational age was admitted to the hospital. Since the age of 12 years, she had suffered recurrent superficial venous thrombosis that had responded on each occasion to conservative therapy consisting of salicylates, lower extremity elevation, and bed rest. At the age of 14 years, she was hospitalized for pneumonia and simultaneously developed left lower extremity pain and swelling. A venogram showed left deep venous thrombosis extending from the popliteal to the common femoral vein. She was treated with heparin for 10 days followed by warfarin sodium therapy to maintain the prothrombin

Table I. Hemostatic evaluation of patient

	Findings	Normal range
Prothrombin time (on warfarin) (sec)	23.5	9.5-12.5
Activated partial prothrombin time (on warfarin) (sec)	46	24-36
Platelets ($\times 10^9/L$)	381	150-400
Antithrombin III activity (%)	109	80-120
Factor V activity (%)	105	50-150
Factor VIII activity (%)	302	50-150
Plasminogen activity	100	80-130
Protein C (antigen) (off warfarin) (%)	93	75-134
Protein S (antigen) (off warfarin) (%)	44	75-160
Prothrombin (antigen) (off warfarin) (%)	97	75-130

time between 18 and 29 seconds (normal, 9.5 to 12.5 seconds). Despite this therapy, a venogram repeated 4 weeks later showed progressive thrombosis with extension of the thrombus into the external iliac vein.

The patient was transferred to the vascular surgery service. A detailed family history identified several family members with recurrent venous thrombosis, including the patient's mother and the following maternal relatives: the grandmother, both aunts, a great aunt, and one first-degree and two second-degree cousins. No paternal relatives had a history of thrombotic or vascular disease. The hemostatic evaluation of the patient is summarized in Table I. A diagnosis of hereditary protein S deficiency was made on the basis of a selective decrease in protein S (American Bioscience Laboratories, Van Nuys, California) in the patient (44%) and her mother (59%). Although the patient was not sexually active at the time the diagnosis was made, she was informed of the teratogenic potential of warfarin sodium and advised to seek medical consultation before planning pregnancy.

One year later, she became pregnant while receiving warfarin therapy; an ultrasound scan confirmed the gestational age of 17 weeks. The patient was counseled that the increased risk of venous thrombosis and pulmonary embolus could be reduced during the preg-

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nancy by heparin therapy. The teratogenic effects of warfarin sodium exposure were discussed. The patient and her family chose elective termination of the pregnancy for social reasons.

One day before the surgical procedure, the patient was admitted, and laminaria tents were inserted in her cervical os in the routine manner. Fresh frozen plasma was administered to reverse the anticoagulant effect of warfarin, and subcutaneous heparin (5000 U every 12 hours) was begun. She was anesthetized by paracervical block, and an uncomplicated dilatation and suction curettage was performed without excessive blood loss. Postoperatively the patient was up and about immediately, and warfarin therapy was resumed. She had no signs of thrombosis when she was discharged on the first postoperative day. She was instructed in the use of foam and condom contraception before she was discharged.

Comment

Protein S, a vitamin K-dependent plasma protein, is a cofactor for protein C, an essential component of the regulatory system of coagulation. Protein S appears to mediate the interaction between protein C and its substrates, factors Va and VIIIa, on phospholipid surfaces. Other disorders that must be considered in the differential diagnosis of recurrent venous thrombosis include protein C deficiency, antithrombin III deficiency, plasminogen deficiency, tissue plasminogen activator deficiency, dysfibrinogenemia, heparin cofactor II deficiency, and lupus coagulants.

The optimal management of protein S deficiency in pregnancy is not established. Of the 12 patients previously described, only three were women. One had venous thrombosis during her single pregnancy; a second had a lifelong recurrent thrombosis and suffered an episode during pregnancy. For the third woman no reproductive information was given. In our patient's family, recurrent thrombosis occurred during pregnancy in six members, all of whom survived.

This high incidence of thrombotic complications during pregnancy suggests that the combination of pregnancy and protein S deficiency may pose risks similar to those known to be associated with pregnancy and antithrombin III deficiency. These patients have been managed by elective abortion or, in pregnancies carried to term, with heparin prophylaxis with satisfactory results. The successful management of our patient by subcutaneous heparin therapy during a high-risk period suggests that this approach may be helpful in protein S deficiency as well. Since warfarin therapy carries a significant fetal risk, with a normal infant expected in only 70% of documented exposures, heparin is the preferred anticoagulant in pregnancy.

Postabortion contraception alternatives are limited by the increased risk of thromboembolism accompanying the use of oral contraceptives and the risk of hemorrhage with use of an intrauterine device in patients receiving anticoagulants. For these reasons a barrier method is preferred.

In summary, protein S deficiency is an uncommon disorder resulting in recurrent venous thrombosis. More detailed family history and wide availability of protein S assays will result in the more efficient identification of affected persons. Although the exact risk of venous thrombosis and embolic complications in pregnant, protein S-deficient patients is not known, appropriate anticoagulant therapy and close surveillance appear to be indicated.

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Evidence of cyclic alterations of thyroid size during the menstrual cycle in healthy women

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Serum levels of thyroxine, triiodothyronine, triiodothyronine resin uptake, thyroxine-binding globulin, thyrotropin, and ultrasonically determined thyroid gland volume were investigated weekly in 11 healthy women during a normal menstrual cycle, and in five healthy men once a week for 5 consecutive weeks. In the men the thyroid volume was unaltered. In the women, however, a mean variation in thyroid volume of approximately 50% between minimum values (15.4 ± 3.1 ml, day 9) and maximum values (24.4 ± 4.8 ml, day 23) was found ($p < 0.01$), although no significant differences in the other thyroid variables could be demonstrated. The menstrual cycle seems to be associated with cyclic alterations of thyroid size in healthy women unexplained by alterations in thyroid function variables. This information should be taken into account when goiter frequency, goitrogenic action of drugs, and goiter treatment effects are evaluated. (AM J OBSTET GYNECOL 1986;155:142-5.)

Key words: Thyroid hormones, thyrotropin, thyroid volume, goiter, menstrual cycle, ultrasound

It is a common clinical experience that many women have noticed variations in neck circumference during the menstrual cycle. No data are available on the influence of the menstrual cycle on thyroid gland size, probably because of the lack of sufficiently sensitive objective methods for this evaluation. The aim of the present study was to determine whether thyroid gland volume, ultrasonically determined,¹ showed any changes during the menstrual cycle in healthy women.

Subjects and methods

Subjects. Included in the study were the following subjects: (1) Eleven women with a median age of 26 years (range, 18 to 42 years) and a median body weight—on day 2 of their menstrual cycle—of 54 kg (range, 52 to 64 kg). All had normal menstrual cycles, which were regular for at least 6 months before the study. The investigations were begun on the second day of menstrual bleeding and terminated on the second day of the following menstrual bleeding. Thyroid volume determination and blood sampling were performed once a week for a whole menstrual cycle (days 2, 9, 16, and 23 and day 2 in the following cycle). (2) Two of these women had their thyroid volume determined almost every day during a complete menstrual

cycle. (3) Five healthy young men with a median age of 32 years (range, 28 to 34 years) and a median body weight of 66 kg (range, 62 to 83 kg) were also studied. Starting on randomly chosen days and continuing once a week for 5 weeks, thyroid gland volume was determined. No blood samples were drawn in these subjects.

Upon entry into the study none of the subjects had known present or past thyroid disease or a detectable goiter. None of the women had been pregnant within the last year, and none of the subjects were taking any kind of medication including oral contraceptives.

Methods. The presence of a goiter was defined as a visible and/or palpable thyroid gland. The ultrasonic scanning procedure and the total volume calculation were performed as previously described with a compound scanner (type 1846, Brüel and Kjaer, Naerum, Denmark).¹ The transducer was a 5.5 MHz type mechanically focused at 1 to 3 cm. The scanning procedure and volume calculation as well as the accuracy and precision of the method were reported previously.¹ Thyroid volume measurements were always performed on the day of thyroid function investigations at 4 PM. All volume determinations in one subject were performed by the same investigator without knowledge of the subject's menstrual cycle phase. The precision of the ultrasonic method was 5.1% in the present study, expressed as the coefficient of variation on double determinations. Measurements of thyroid volume in four normal men three times a day on 3 consecutive days showed a mean coefficient of variation of 5.7% in day-to-day measurements and of 4.8% in measurements within the same day.

Serum thyroxine, triiodothyronine, triiodothyronine

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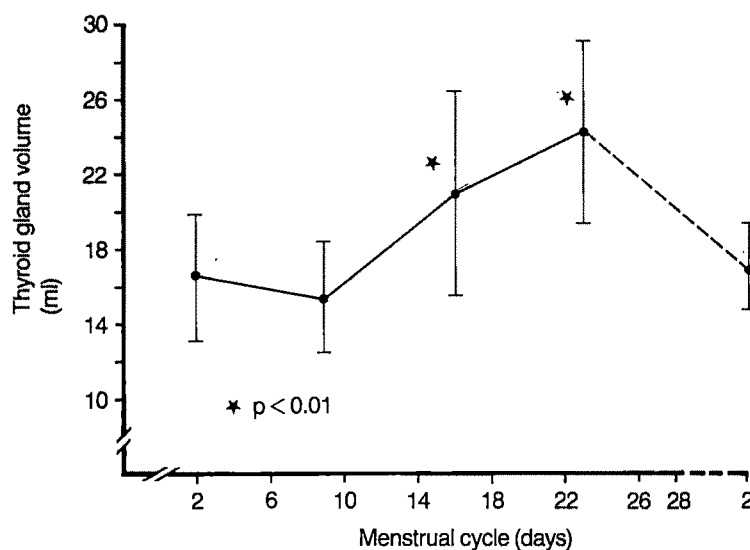


Fig. 1. Thyroid gland volume (mean $\pm$ SD) in 11 healthy women during a normal menstrual cycle. Investigations were performed once a week, and mean thyroid volume was significantly higher on days 16 and 23 than on days 2 and 9 ($p < 0.01$).

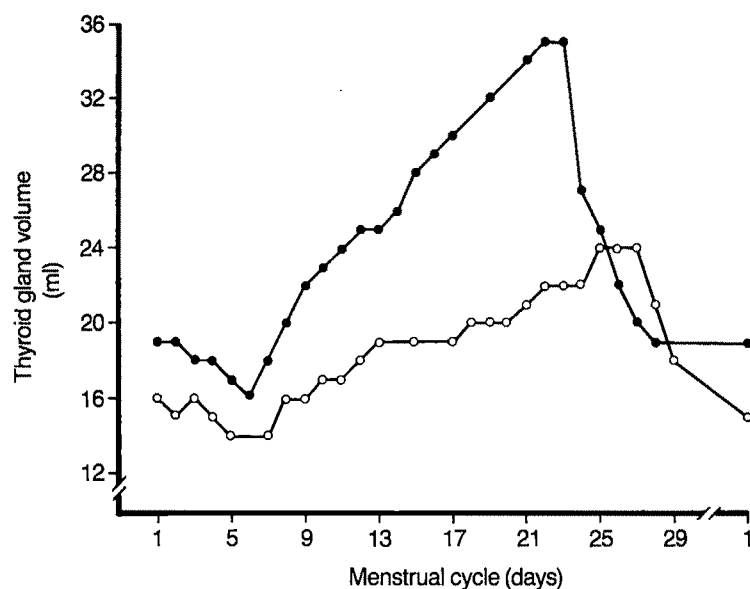


Fig. 2. Thyroid gland volume determined almost every day during a normal menstrual cycle in two healthy women.

resin uptake, and thyrotropin were determined as previously described.^{1,2} The interassay variation for serum thyrotropin determination was 7.2%, and the intra-assay variation was 5.8% in the range of serum thyrotropin measured. The detection limit was 0.3 μ U/ml. Thyroxine-binding globulin was measured by rocket immunoelectrophoresis.³ The free thyroxine index and free triiodothyronine index were calculated as total hormone concentration times triiodothyronine resin uptake and given in arbitrary units. Serum was stored at -20° C, and analyzed in random order in the same assay series.

Results were examined by one-way analysis (Friedman's test for related samples). If this test indicated that some differences existed, multiple comparisons were performed according to Conover.⁴ Probability values of <0.05 were regarded as significant.

Results

Among the women the median body weight was 54 kg (range, 52 to 64 kg) on day 2 and 56 kg (range, 54 to 66 kg) on day 23 ($p < 0.05$). A significant increase in thyroid gland volume during a normal menstrual cycle was observed (Fig. 1). Thus mean thyroid volume

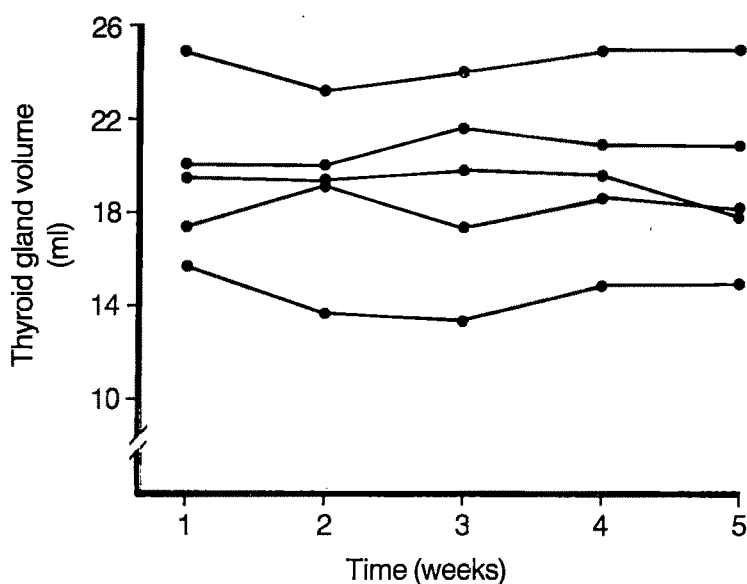


Fig. 3. Thyroid gland volume determined once a week for 5 consecutive weeks in five healthy men.

Table I. Thyroid function variables during the normal menstrual cycle in 11 healthy women (mean $\pm$ SD)

Cycle day	Thyroxine (nmol/L)	Triiodothyronine (nmol/L)	Triiodothyronine resin uptake (arbitrary units)	Thyroxine-binding globulin (arbitrary units)	Free thyroxine index (arbitrary units)	Free triiodothyronine index (arbitrary units)	Thyrotropin (μ U/ml)
2	95.5 $\pm$ 13.8	2.1 $\pm$ 0.4	1.00 $\pm$ 0.09	104.7 $\pm$ 14.2	96.9 $\pm$ 15.5	2.12 $\pm$ 0.56	1.9 $\pm$ 0.8
9	102.7 $\pm$ 10.8	2.1 $\pm$ 0.3	1.01 $\pm$ 0.02	107.9 $\pm$ 14.0	103.5 $\pm$ 13.3	2.15 $\pm$ 0.30	2.3 $\pm$ 0.7
16	94.6 $\pm$ 7.9	2.1 $\pm$ 0.5	1.00 $\pm$ 0.02	107.2 $\pm$ 12.1	94.3 $\pm$ 11.3	2.12 $\pm$ 0.49	2.1 $\pm$ 0.8
23	95.6 $\pm$ 11.3	2.1 $\pm$ 0.3	0.99 $\pm$ 0.02	105.9 $\pm$ 14.4	94.7 $\pm$ 14.3	2.05 $\pm$ 0.23	2.4 $\pm$ 1.0
2 (of following cycle)	96.2 $\pm$ 9.4	2.2 $\pm$ 0.4	0.99 $\pm$ 0.04	105.8 $\pm$ 13.7	96.4 $\pm$ 15.0	2.17 $\pm$ 0.49	2.0 $\pm$ 0.8

varied approximately 50% between minimum (15.4 $\pm$ 3.1 ml, day 9) and maximum values (24.4 $\pm$ 4.8 ml, day 23) ($p < 0.01$, Fig. 1). Values were significantly higher on day 16 and 23 than on day 2 and 9 ($p < 0.01$, Fig. 1). No significant difference was found between thyroid volume at the start (day 2) or at the termination of the investigation (day 2 of next cycle). None of the women had a clinically detectable goiter at any time during their menstrual cycle. In the two women investigated every day during a whole cycle, maximum thyroid volume was observed on days 22 to 23 and 25 to 27, respectively (Fig. 2). No such pattern could be observed in the men (Fig. 3). The mean alterations in thyroid volume in the men were of the same magnitude (4.6%) as the previously reported day-to-day variation of this parameter (5.7%).

No significant alterations in any of the other thyroid variables could be demonstrated in the women during the menstrual cycle (Table I).

Comment

The present study verifies the experience of a menstrual cycle-related cyclic alteration of thyroid size. The reason for this information not hitherto being available

is best explained by the lack of sufficiently sensitive methods for this evaluation. Furthermore, none of the women had a clinically detectable goiter at any time during the investigation, and thyroid volume alterations mainly occurred within the normal range.¹ Thyroid volume is closely related to body weight,¹ so an increase in body weight in the second half of the menstrual cycle could in part be the explanation for this increase in thyroid volume. The present study does not allow speculations on the nature of this increase. However, the abrupt return within a few days to normal size around the onset of the menstrual bleeding makes it most plausible to speculate regarding altered vascularity of the thyroid gland rather than hypertrophy.

It has been reported that female rats have cyclic variations in thyrotropin and thyroid activity correlating with the estrous cycle.⁵ Some have found an increase in thyroid weight (hyperplasia) after administration of estrogen.⁶ Prolonged treatment, however, resulted in involution of the thyroid gland. Others have not found such an increase in thyroid weight.⁷ Previous studies, also in animals, have shown that the action of estrogen on the thyroid was not mediated via the pituitary, and it was assumed that estrogen acted directly on the thy-

roid.^{8,9} The present study and other studies^{10,11} support this concept, since no alterations in serum thyrotropin levels could be demonstrated. In addition Carlson et al.¹² found unaltered basal and thyrotropin-releasing hormone-stimulated serum thyrotropin values in healthy males treated with estrogen. It can, however, not be ruled out that the use of a new ultrasensitive thyrotropin method and/or more frequent blood sampling could have disclosed significant alterations in serum thyrotropin level.

It is well established that estrogens—synthetic as well as genuine—stimulate the production of thyroxine-binding globulin.¹³ This normally leads to an increase in serum thyroxine and triiodothyronine levels but free thyroxine and free triiodothyronine levels are usually unchanged. This mechanism of estrogen-stimulated thyroxine-binding globulin production has been suggested as partly responsible for the goitrogenic effect of pregnancy.¹⁴ The present study was, however, not able to demonstrate alterations in serum thyroxine-binding globulin concentration or the other thyroid variables except thyroid volume. Furthermore, recent investigations have questioned this,^{15,16} and it is important to stress that no objective studies addressing the effect of pregnancy on thyroid size are available. In acute reversible liver disease (hepatitis) there is a reversible increase in serum thyroxine-binding globulin (due to destruction of hepatocytes) and thyroid volume, but serum thyrotropin level is unchanged.¹⁷ The mechanism underlying thyroid volume alterations during and after acute liver disease was not clarified, possibly again the result of infrequent blood sampling and/or lack of a supersensitive thyrotropin method.

The menstrual cycle is associated with changes in a number of hormonal systems such as aldosterone,¹⁸ prolactin,¹⁹ catecholamines,²⁰ progesterone,²¹ and vasopressin,²² but the interrelationship between these hormonal systems and thyroid function and size is not clarified.

In conclusion, a cyclic alteration in thyroid size unexplained by alterations in thyroid hormones or thyrotropin has been demonstrated. If these changes in thyroid gland volume during a normal menstrual cycle are not considered, they might constitute a pitfall in the evaluation and comparison of the goiter frequency in different populations. Furthermore, if these alterations are also present in subjects with goiter, the evaluation of e.g. treatment results after thyroxine²³ or triiodothyronine²⁴ treatment of diffuse nontoxic goiter may be heavily biased if not corrected for this finding.

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Dietary fatty acids and platelet thromboxane production in puerperal women and their offspring

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The effects of the ratio of polyunsaturated to saturated fatty acids in the diet of puerperal women on maternal and neonatal fatty acids and thromboxane A_2 synthesis were studied in 91 mother-infant pairs on the fourth to fifth day after delivery. The mothers received a diet with either a low (0.1) or a high (1.5) polyunsaturated to saturated fatty acid ratio for 4 to 5 days. The fatty acid composition in maternal and neonatal serum and in breast milk was determined in 34 mother-infant pairs from both dietary groups. The capacity of the platelets to produce thromboxane B_2 , a stable metabolite of thromboxane A_2 , was determined in another 57 mother-infant pairs. The percentage of linoleic acid was increased in maternal and neonatal sera as well as in the breast milk in the group with a high dietary polyunsaturated to saturated fatty acid ratio, whereas the content of arachidonic acid was increased only in maternal serum. This was accompanied by increased platelet thromboxane B_2 production in the mothers, and maternal and neonatal thromboxane B_2 synthesis correlated significantly with each other ($r = 0.43$, $p < 0.05$). This finding may provide therapeutic possibilities in the future. (AM J OBSTET GYNECOL 1986;155:146-9.)

Key words: Dietary fatty acids, thromboxane A_2 , thromboxane B_2 , puerperal women, newborn infants

Arachidonic acid (20:4w6) is the immediate precursor of dienoic prostaglandins and is synthesized from linoleic acid (18:2w6) in the human body.^{1,2} Since humans are unable to synthesize linoleic acid, they are entirely dependent on the dietary supply of linoleic acid and arachidonic acid.² The major prostaglandin produced by the platelets is thromboxane A_2 , which is a potent proaggregatory and vasoconstrictory agent that is synthesized and released during platelet aggregation.^{3,4} In puerperal women and neonates the platelet thromboxane A_2 production is higher than that in non-pregnant subjects, although the reasons for this phenomenon are unknown.^{3,6} On the other hand, the fatty acid composition of the diet may influence thromboxane A_2 synthesis,⁷ and dietary polyunsaturated fatty acids have been reported to decrease the thromboxane A_2 synthesis in vitro.⁷ We compared the effects of a maternal diet with either a low (0.1) or a high (1.5) ratio of polyunsaturated to saturated fatty acids (P/S ratio)

on fatty acid composition in maternal and neonatal serum and breast milk and on the maternal and neonatal platelet thromboxane A_2 production.

Subjects and methods

Subjects. Ninety-one mothers and their healthy neonates on two postnatal wards volunteered for this study with the approval of the local ethical committee. During a 3-week period the mothers on one puerperal ward received an ordinary hospital diet with a P/S ratio of 0.1, while the mothers on the other ward had a diet rich in vegetable oils, chicken meat and fish, vegetables, and fruits (P/S ratio 1.5). During a second 3-week period the diets on the wards were switched. The total energy intake was kept on the same level in both diets (2000 to 2200 kcal/day). The infants of mothers with ordinary and experimental diets had similar gestational ages (38.5 ± 1.2 and 38.9 ± 1.0 weeks, mean $\pm$ SD, respectively) and birth weights (3300 ± 640 and 3510 ± 550 gm, mean $\pm$ SD, respectively). The infants were breast-fed normally, and if additional breast milk was needed, it was collected from the mothers using the same diet. Samples of maternal and neonatal serum from a peripheral vein and breast milk were collected for fatty acid analysis from 34 mother-infant pairs on the fourth or fifth day of dietary intervention. On the same days maternal and neonatal serum samples by venipuncture were taken from another 57 mother-infant pairs for thromboxane A_2 determinations (26 on

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Table I. The percentages of polyunsaturated and saturated fatty acids in maternal and neonatal serum and breast milk in 34 mother-infant pairs after 4 to 5 days of use of an ordinary hospital diet with P/S ratio of 0.1 or an experimental diet with P/S ratio of 1.5

Fatty acid	Mother		Breast milk		Infant	
	Ordinary diet (mean $\pm$ SD)	Experimental diet (mean $\pm$ SD)	Ordinary diet (mean $\pm$ SD)	Experimental diet (mean $\pm$ SD)	Ordinary diet (mean $\pm$ SD)	Experimental diet (mean $\pm$ SD)
14:0	1.7 $\pm$ 0.5	1.2 $\pm$ 0.3*	9.1 $\pm$ 2.4	8.7 $\pm$ 0.4	2.0 $\pm$ 0.7	1.8 $\pm$ 0.7
16:0	24.1 $\pm$ 1.5	23.1 $\pm$ 1.8	29.2 $\pm$ 2.4	24.3 $\pm$ 2.4†	27.6 $\pm$ 1.7	26.1 $\pm$ 2.2‡
18:0	6.5 $\pm$ 0.7	6.2 $\pm$ 0.5	9.4 $\pm$ 0.9	7.4 $\pm$ 1.0†	8.0 $\pm$ 0.3	7.5 $\pm$ 0.4†
18:2	26.6 $\pm$ 5.5	32.3 $\pm$ 4.9†	9.1 $\pm$ 3.4	21.3 $\pm$ 5.6†	14.1 $\pm$ 2.6	18.4 $\pm$ 5.1*
18:3w6	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1
18:3w3	0.6 $\pm$ 0.1	0.4 $\pm$ 0.1*	0.6 $\pm$ 0.2	0.7 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
20:3w6	1.7 $\pm$ 0.2	1.9 $\pm$ 0.3‡	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1†	1.5 $\pm$ 0.2	1.5 $\pm$ 0.3
20:4w6	4.5 $\pm$ 0.8	5.3 $\pm$ 1.3‡	0.6 $\pm$ 0.1	0.7 $\pm$ 0.2	7.2 $\pm$ 1.2	7.9 $\pm$ 1.4
20:5w3	0.6 $\pm$ 0.2	0.5 $\pm$ 0.2	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1
22:6w3	2.1 $\pm$ 0.5	2.1 $\pm$ 0.4	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	2.7 $\pm$ 0.6	2.7 $\pm$ 0.5

* $p < 0.01$ in comparison with the values of the ordinary hospital diet.† $p < 0.001$ in comparison with the values of the ordinary hospital diet.‡ $p < 0.05$ in comparison with the values of the ordinary hospital diet.**Table II.** Platelet thromboxane B_2 production in 57 mother-infant pairs with ordinary hospital diet (P/S ratio of 0.1) or with experimental diet (P/S ratio of 1.5)

Thromboxane B_2	Dietary group		Difference	Confidence limits	p Value
	Ordinary ($n = 26$) (mean $\pm$ SD)	Experimental ($n = 31$) (mean $\pm$ SD)			
Maternal serum (ng/ml)	112.5 $\pm$ 115.8	205.6 $\pm$ 132.2	93.1	90.5-95.7	<0.025
Neonatal serum (ng/ml)	189.2 $\pm$ 106.1	233.4 $\pm$ 104.2	44.2	43.1-45.3	NS

the standard diet and 31 on the experimental diet). None of the mothers in the study had taken drugs known to interfere with prostaglandin synthesis during 10 days preceding the study.

Methods and sampling

Fatty acid analysis. Aliquots of milk or serum (100 to 200 μ l) were extracted with chloroform-methanol (1:1, v/v). The extract was purified, evaporated, and saponified in potassium hydroxide-ethanol (0.5 mol/L), as described in detail previously.⁸ After extraction of the nonpolar material with petroleum ether (boiling point 60° to 80° C), the lower phase was acidified with hydrochloric acid and the fatty acids were extracted into petroleum ether. The solvent was evaporated and the fatty acids were esterified with hydrochloric acid-methanol.⁸ The methyl esters were analyzed in a Hewlett-Packard 6880A gas chromatograph with the use of an open tubular fused silica column 25 m long and 0.32 mm inside diameter coated with OV-351 (Orion Analytica, Espoo, Finland) and a flame ionization detector. All peaks corresponding to fatty acids from 14:0 to 22:6 were quantified with a Hewlett-Packard 6880A

integrator. The results were expressed as percentages of the total area of all identified fatty acid peaks.

Thromboxane A_2 measurement. To study the ability of the platelets to generate thromboxane A_2 during spontaneous clotting, a blood sample (3 to 5 ml) was drawn from both mothers and infants into a dry test tube and allowed to clot at 37° C for 60 minutes. The concentration of thromboxane B_2 , a hydration product of thromboxane A_2 , in serum was then measured radioimmunologically.⁹

Statistical analyses. Because of the abnormal distribution of the thromboxane B_2 values, thromboxane B_2 data were transformed logarithmically and the significances of the differences were tested with Student's t test. Pearson's correlation coefficient was calculated between the thromboxane B_2 values of the mothers and their infants. The results are expressed as means and SDs.

Results

A maternal diet with a high P/S ratio was accompanied with an increased content of linoleic acid, dihomono-

γ -linoleic acid, and arachidonic acid in the serum of the mothers and increased proportions of linoleic acid and dihomogamma-linoleic acid in the breast milk (Table I). Linoleic acid was also increased in neonatal serum (Table I).

Ingestion of a diet with a high P/S ratio resulted in increased production of thromboxane B_2 by maternal but not neonatal platelets (Table II), although maternal and neonatal thromboxane B_2 production capacities were related to each other ($r = 0.43$, $p < 0.05$). A similar trend was also seen in mother-infant pairs with ordinary diet ($r = 0.38$, $0.1 > p > 0.05$).

Comment

Dietary manipulation of the supply of fatty acids may have important clinical consequences,¹ which also involve the breast-fed infants. We present evidence that a short-term (4 to 5 days) change in the maternal dietary P/S ratio is already reflected in the fatty acid composition of breast milk and/or neonatal serum. This is in agreement with previous data showing that administration of polyunsaturated fatty acids to mothers increases their percentages in serum lipids but decreases the content of cholesterol in the maternal and neonatal blood.^{1, 10, 11} These changes, on the other hand, could be important in the prevention of vascular atherogenesis, possibly through changes in the prostanoid biosynthesis.¹²

In our study a rise in the dietary P/S ratio from 0.1 to 1.5 increased the proportions of the precursors of dienoic prostaglandins (linoleic acid, dihomogamma-linoleic acid, and arachidonic acid), whereas the concentration of α -linoleic acid (18:3w3) was decreased in the mothers. Because the latter can also be converted to eicosapentaenoic acid (20:5w3) in man,¹³ its reduction may cause a decrease in the synthesis of trienoic prostaglandins.¹⁴ Because of the competition between the syntheses of series 2 and 3 prostaglandins, this change should result in increased production of series 2 prostanoids, and, indeed, maternal platelet thromboxane B_2 production was increased during the dietary intervention. On the other hand, there was no decrease in the proportion of eicosapentaenoic acid in our experiment (Table I). In the infants the change of maternal diet resulted in an increase in the serum linoleic acid, whereas the thromboxane B_2 production was unchanged. There was, however, a correlation between maternal and neonatal thromboxane B_2 productions, which suggests that perhaps a more profound dietary intervention could have also increased the neonatal thromboxane B_2 synthesis.

Previous studies on nonpuerperal subjects have shown that platelet aggregation decreases after ingestion of diets rich in polyunsaturated fatty acids, espe-

cially linoleic acid.^{15, 16} Our finding of an increased production of proaggregatory and vasoconstrictory thromboxane A_2 in puerperal women with a high dietary P/S ratio is in apparent discrepancy with these results. There may be several explanations for the discrepancy: (1) The diets in different studies have not been standardized. Varying amounts of fatty acids other than linoleic acid, for instance, arachidonic acid and eicosapentaenoic acid, may lead to different results in both platelet aggregation and thromboxane A_2 production. (2) The production of thromboxane A_2 may not relate directly to the aggregation of the platelets. Other diet-dependent factors, such as a change in membrane fluidity, production of the antiaggregatory prostaglandin E_1 from dihomogamma-linoleic acid, and the balance between thromboxane A_2 and its biologic antagonist prostacyclin, may influence the aggregation process.^{1, 2} (3) We studied puerperal women, whose thromboxane B_2 production is increased⁵ and who may respond differently to the dietary changes in P/S ratio. These changes may also be sex-dependent, since such a dietary manipulation decreases the thromboxane B_2 production in men but tends to increase it in women.¹²

The clinical significance of the increased thromboxane A_2 synthesis remains unknown. Prostanoids are hemodynamically important during the early neonatal period.⁶ It is possible that their metabolism could be affected by changing the supply of fatty acids to the newborn infant through alterations in the maternal diet, as is evident from our data. Thus maternal dietary manipulation may offer new possibilities for treatment and prevention of various neonatal disorders.

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The use of the nonstress test in patients with premature rupture of the membranes

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The value of the nonstress test in predicting the outcome of infection was determined by a retrospective analysis of 127 consecutive high-risk patients who presented with premature rupture of the membranes and no clinical signs of infection or labor. The last study performed within 48 hours of delivery was used for comparison with the outcome of pregnancy. The relationship between nonstress test results and the outcome of pregnancy, as reflected by the development of clinical amnionitis and/or neonatal sepsis, was determined. The sensitivity and specificity of the nonstress test in predicting infection outcome in patients with premature rupture of the membranes were 78.1% and 86.3%, respectively. These data suggest that the nonstress test is a useful tool for evaluating patients with premature rupture of the membranes. (*AM J OBSTET GYNECOL* 1986;155:149-53.)

Key words: Nonstress test, premature rupture of membranes

Premature rupture of the membranes is one of the most common complications of pregnancy. It occurs in approximately 10% of all pregnancies¹ and is associated with an increased incidence of perinatal death,² fetal distress during labor,³ and infection (maternal and fetal).⁴ Since the contraction stress test is contraindicated in the presence of ruptured membranes, antepartum fetal evaluation of these patients has been confined solely to nonstress testing. The value of the nonstress test has been well documented in high-risk patients with

intact membranes^{5,6}; however, its value in the antepartum fetal surveillance of patients with premature rupture of the membranes has not been determined accurately. The fetal biophysical profile has been described⁷ recently by the authors as an early predictor of impending infection in patients with premature rupture of the membranes. In that report⁷ a tendency for a higher incidence of infection with nonreactive nonstress testing was observed.

Since the nonstress test is most frequently used as a primary antepartum fetal surveillance test, the present retrospective study was undertaken to determine the value of the nonstress test in evaluating patients who presented with premature rupture of the membranes and no clinical signs of infection or labor. Measures of pregnancy outcome included the presence of clinical amnionitis, possible neonatal sepsis, and neonatal sepsis.

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Table I. Mean gestational ages (on admission and at delivery), birth weights, Apgar scores <7, umbilical cord pH, and nonstress testing results in cases of infection versus those without infection.

Patients (n = 127)	Gestational age on admission (mean $\pm$ 2 SD) (wk)	Gestational age at delivery (mean $\pm$ 2 SD) (wk)	Apgar scores <7			
			1 min		5 min	
			n	%	n	%
Infected (n = 32)	29.7 $\pm$ 6.4	30.3 $\pm$ 5.9	21	65.6	9	28.1
Noninfected (n = 95)	32.1 $\pm$ 5.6	32.7 $\pm$ 5.6	22	23.2	1	1.1
p Value	<0.001	<0.001	<0.001		<0.001	

NSTs = Nonstress tests; NS = not significant.

Material and methods

All the records of patients who were admitted with premature rupture of the membranes from July 1, 1983, to May 30, 1985, were reviewed. A total of 127 patients were included in this retrospective study. The majority of patients (121 of 127, 95.3%) were transferred to The University of Connecticut Health Center because of preterm rupture of the membranes. Only patients with singleton pregnancies and a gestational age of ≥ 25 weeks who presented with premature rupture of the membranes were included. These women were not in labor. Patients with signs of infection, labor, bleeding, or fetal distress were excluded. Women receiving tocolytic agents or who had complications such as diabetes, hypertension, or intrauterine growth retardation were also excluded. In all patients rupture of the membranes was diagnosed by the presence of pooled fluid at the time of sterile speculum examination that exhibited a ferning pattern and had an alkaline pH determination. Bimanual pelvic examinations were not done after admission to our institution. Routine vital signs were obtained on admittance, and electronic fetal heart rate monitoring was used to detect signs of fetal distress and document any uterine activity. If there was no evidence of labor, fetal distress, or infection, an ultrasound evaluation was performed to confirm the gestational age and determine a fetal biophysical profile score.^{7,8} The nonstress test was performed as part of the fetal biophysical profile according to a technique previously described.⁸ All patients were observed until delivery and were managed conservatively with bed rest and bathroom privileges. Corticosteroid therapy was administered in 85 of 127 patients. Temperatures were taken four times a day and white blood cell counts were performed daily. Bimanual pelvic examinations were performed only if the patient was believed to be in active labor or a decision had been made to induce labor. The nonstress tests were repeated every 24 to 48 hours if patients remained undelivered. Nonreactive nonstress tests were followed by either repeat testing or determination of the fetal biophysical profile. Indications for delivery included labor, diagnosed clinical amnionitis, persistent spontaneous variable decelerations (moderate to severe) on electronic fetal heart rate

monitoring, or gestational age >37 weeks after a 24-hour period of observation. In some cases a biophysical profile score of ≤ 7 was also used as an indication for delivery.^{7,8} Antibiotics were administered only after clamping of the umbilical cord. In all patients the final nonstress test was within 48 hours of delivery.

On admittance to the neonatal intensive care unit, bacterial cultures were obtained from all infants (blood, urine, cerebrospinal fluid, tracheal fluid, nasogastric fluid, and external ear). All neonates at <34 weeks' gestation received prophylactic antibiotics, which were discontinued when the cultures demonstrated no growth. When neonatal sepsis or possible neonatal sepsis was diagnosed, the antibiotics were continued for approximately 7 to 10 days.

For the purpose of this report a reactive nonstress test was defined as one with two or more fetal heart rate accelerations of at least 15 bpm in amplitude and at least 15 seconds' duration in a 20-minute period. A nonreactive nonstress test was defined as the presence of one or no accelerations in a 20-minute period. The diagnosis of clinical amnionitis, possible neonatal sepsis, and neonatal sepsis was based on criteria previously described.⁷ For the purpose of this study infection outcome was defined as the presence of clinical amnionitis, possible neonatal sepsis, or neonatal sepsis.

The postdelivery collection data included Apgar scores, birth weights, cord blood pH (umbilical arterial and umbilical venous), route of delivery, neonatal septic workup, and perinatal deaths. The last study within 48 hours of delivery was compared with the outcome of pregnancy, as reflected by the development of amnionitis, possible neonatal sepsis, and neonatal sepsis (infection outcome). The data were also analyzed according to the longitudinal trend of the nonstress results from admission to delivery and compared with the outcome of pregnancy.

Statistical analysis was performed by use of χ^2 or Student's t test when appropriate.

Results

Three hundred forty-five nonstress tests were performed in the 127 patients who fulfilled the inclusion criteria (2.7 examinations per patient). The most ex-

Birth weight (mean $\pm$ 2 SD) (gm)	Cord pH (mean $\pm$ 2 SD)		Reactive NSTs		Nonreactive NSTs	
	Arterial	Venous	n	%	n	%
1465 $\pm$ 1047	7.29 $\pm$ 0.14	7.34 $\pm$ 0.11	7	21.8	25	78.1
1907 $\pm$ 1184	7.30 $\pm$ 0.11	7.35 $\pm$ 0.11	82	86.3	13	13.7
<0.001	NS	NS	<0.001		<0.001	

aminations done on one patient was 25. The mean gestational age on admission was 31.5 weeks (range 25 to 41 weeks) and the mean gestational age at delivery was 32.1 weeks (range 25.5 to 41.2 weeks). Of the 127 patients, 121 (95.3%) were delivered preterm (<37 completed weeks) and six (4.6%) were delivered at term.

Of the 127 patients 32 (25.1%) developed infection. The diagnoses in these 32 patients were amnionitis (19, 14.9%), possible neonatal sepsis (17, 13.3%), and neonatal sepsis (9, 7%). Some patients had more than one diagnosis. On the basis of the final study before delivery 89 (70%) patients had reactive nonstress tests and 38 (30%) had nonreactive nonstress tests.

Table I illustrates the mean gestational ages, birth weights, Apgar scores <7, umbilical cord pH, and nonstress test results in cases of infection versus those without infection. Of the 32 patients who developed infection 25 (78.1%) had nonreactive nonstress tests and the remaining seven (21.8%) had reactive tests. Of the 95 who did not develop infection, 13 (13.7%) had nonreactive nonstress tests and the remaining 82 (86.3%) had reactive tests. The infected group had a significantly lower mean gestational age (on admission and at delivery) and birth weight ($p < 0.001$) but a significantly higher incidence of low Apgar scores and nonreactive nonstress tests ($p < 0.001$). Table II illustrates the relationship between the nonstress test results and infection outcomes of the entire group. The sensitivity was 78.1% and specificity was 86.3%. Since the higher incidence of nonreactive nonstress tests in the infected group could be contributed to lower gestational age, the results were also analyzed according to gestational age groups (≤ 32 weeks' and > 32 weeks' gestation). The relationship between nonreactive nonstress testing and infection outcome for different gestational age groups is illustrated in Table III. As can be seen the incidence of nonreactive nonstress tests was significantly higher in patients who developed infection before as well as after 32 weeks' gestation. Table IV demonstrates the efficacy of the nonstress test to predict infection according to gestational age groups.

The data were also analyzed according to the longitudinal trend of the nonstress test results. Of the 127 patients, 71 (56%) had serial nonstress tests and 56 (44%) had only one test performed because they were

delivered within the first 24 to 48 hours of admission. Nineteen patients initially had a reactive nonstress test but subsequently had a nonreactive test, and 17 of these (89.5%) developed infection. There were five patients who had persistently nonreactive nonstress testing from admission to delivery and four of these (80%) developed infection. There were 43 patients with persistently reactive nonstress testing from admission to delivery and only two of these (4.6%) developed infection. There were four patients who initially had nonreactive nonstress testing which subsequently was converted to reactive testing and none of these developed infection. Of the 56 patients who had only one test before delivery, there were 42 with a reactive nonstress test and only three of these (7.1%) developed infection; the remaining 14 had nonreactive nonstress tests and six of these (43%) developed infection.

There were a total of nine instances of positive neonatal cultures. Of these, six were recovered from the blood (three group B streptococci, one pneumococcus, one *Bacillus* species, and one *Escherichia coli*), two from the urine (one positive for group B streptococci and one positive for peptostreptococci), and one from cerebrospinal fluid (group B streptococci). There were only three neonatal deaths in the population tested. Two of these infants were delivered at 26 weeks' gestation because of amnionitis and severe variable decelerations, and one, who was delivered at 32 weeks' gestation, died of group B streptococcal sepsis.

Comment

Premature rupture of the membranes constitutes one of the most common complications of pregnancy.¹ When it occurs at term the majority of investigators recommend prompt delivery because of an increased incidence of amnionitis with prolonged rupture of the membranes.⁹ However, this action may result in an increased incidence of primary cesarean section for failed induction of labor or cephalopelvic disproportion.⁴ When premature rupture of the membranes occurs in preterm gestation, the management is even more controversial. In preterm gestations immediate delivery of the fetus carries a significant risk of hyaline membrane disease, which still remains the major contributor to neonatal morbidity and mortality; therefore conser-

Table II. Relationship between nonstress test results and infection outcome (entire group)

NSTs	Infection outcome*	Diagnoses (in 32 infected patients)			Noninfection outcome
		Amnionitis	Possible neonatal sepsis	Neonatal sepsis	
Nonreactive NSTs (n = 38)	25	14	15	7	13
Reactive NSTs (n = 89)	7	5	2	2	82
Total (n = 127)	32	19	17	9	95

NSTs = Nonstress tests. Sensitivity = 78.1% (25 of 32); specificity = 86.3% (82 of 95); positive predictive value = 65.7% (25 of 38); negative predictive value = 92.1% (82 of 89). Sensitivity for amnionitis = 73.6% (14 of 19); possible neonatal sepsis = 88.2% (15 of 17); neonatal sepsis = 77.7% (7 of 9).

*Infection outcome includes 32 patients who may have more than one diagnosis.

Table III. Relationship between nonstress test results and infection outcome according to gestational age groups

	Nonreactive NSTs	
	no.	%
<i>Patients with gestational age ≤32 wk (n = 55)</i>		
Infected (n = 21)	16/21	76.1
Noninfected (n = 34)	9/34	26.5
p Value	<0.001	
<i>Patients with gestational age >32 wk (n = 72)</i>		
Infected (n = 11)	9/11	81.8
Noninfected (n = 61)	4/61	6.6
p Value	<0.001	

NSTs = Nonstress tests.

vative management has been advocated by some investigators.¹⁰ However, the conservative approach carries an increased risk of maternal-fetal infection and cord prolapse. Other management protocols involve the use of amniocentesis^{11,12} to detect not only the fetus with a mature lung profile but also the fetus most likely to develop sepsis. The problems associated with this approach have been the inability to obtain fluid in almost half of these patients,¹¹ the invasiveness of the procedure, and the lack of strong correlation between the presence of bacteria in the amniotic fluid and fetal sepsis.^{11,12} In summary, a uniform approach to the management of patients with premature rupture of the membranes has not yet been elucidated.

It is our belief that any management protocol in patients with premature rupture of the membranes and no signs of labor or infection should consider the condition of the fetus and therefore should include fetal surveillance. The contraction stress test has been avoided in the evaluation of these patients because of the theoretical risk of initiating labor. Although the nonstress test has been the only means of antepartum fetal evaluation in patients with premature rupture of the membranes, its role has not been adequately defined in evaluating these patients. We recently described the fetal biophysical profile as an early predictor

Table IV. Comparison of the efficacy of nonstress test in prediction of infection according to gestational age groups

Gestational age	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
≤32 wk	76.1	58.8	61.5	80.0
>32 wk	81.8	95.0	75.0	96.6
Entire group (25-41 wk)	78.1	86.3	65.7	92.1

Nonstress test, premature rupture of membranes

of impending infection in patients with premature rupture of the membranes. In that report,⁷ which involved the use of frequent biophysical profile determinations (every 24 to 48 hours), the best predictor of impending infection was found to be the overall biophysical score. Of the individual fetal biophysical components the nonstress test was found to be the most important in predicting infection versus noninfection outcomes. Since the nonstress test is the most widely used as a primary antepartum fetal surveillance test, we undertook this retrospective study to determine the value of serial nonstress testing assessment (every 24 to 48 hours) in evaluating patients with premature rupture of the membranes with no signs of infection or labor.

According to our data, patients who developed infection had a 78.1% incidence of nonreactive nonstress testing and a 21.8% incidence of reactive nonstress testing. Patients who did not develop infection had a 13.7% incidence of nonreactive nonstress testing and an 86.3% incidence of reactive nonstress testing (Table I). The sensitivity, specificity, positive predictive value, and negative predictive value of the nonstress test in predicting infection outcome for all gestational ages were found to be 78.1%, 86.3%, 65.7%, and 92.1%, respectively (Table II). Nonreactive nonstress testing was encountered in 14 of 19 cases of clinical amnionitis (sensitivity, 73.6%), 15 of 17 cases of possible neonatal sepsis (sensitivity, 88.2%), and seven of nine cases of neonatal sepsis (sensitivity, 77.7%). Since preterm gestations <32

weeks are normally associated with an increased incidence of nonreactive nonstress testing,¹³ the value of the nonstress test in predicting infection was also analyzed according to different gestational age groups on the basis of the gestational age at delivery (>32 weeks and ≤ 32 weeks) (Tables III and IV).

In gestational ages of ≤ 32 weeks, 76.1% of the infected cases had nonreactive nonstress tests versus 26.5% of the noninfected cases. In gestational ages >32 weeks the prediction of infection outcome was even better, since 81.8% of the infected cases, versus 6.6% of the noninfected cases, had nonreactive nonstress tests. The sensitivity, specificity, positive predictive value, and negative predictive value of the nonstress test in predicting infection in gestational ages >32 weeks were 81.8%, 95.0%, 75.0%, and 96.6%, respectively (Table IV). The group of patients with an initial reactive nonstress test that subsequently had a nonreactive test as well as the group with a persistently nonreactive nonstress test since admission had the highest infection rates (89.5% and 80%, respectively).

Frequent nonstress testing (every 24 hours) should be part of the management scheme for patients with premature rupture of the membranes. According to our data the group of patients with an initial reactive nonstress test with conversion to a nonreactive test in subsequent testing is the one most likely to develop infection. The presence of a reactive nonstress test is reassuring against infection (high negative predictive value). The presence of persistent nonreactive nonstress testing, even in a very premature gestation, is associated with a high incidence of infectious outcome and its presence should be further evaluated. In our institution the presence of nonreactive nonstress testing is followed up with determination of the fetal biophysical profile. A fetal biophysical profile score of ≤ 7 , with a nonreactive nonstress test and absence of fetal breathing movements, is highly predictive of impending fetal infection⁷ and has been used by us as an indication for immediate delivery. The mechanism by which impending infection causes nonreactive nonstress testing is unknown. Our speculation is that infection in early stages may increase fetal oxygen de-

mands and cause tissue hypoxia and therefore malfunction of the central nervous system centers that control fetal heart rate reactivity.

In conclusion frequent (every 24 hours) nonstress tests in patients with premature rupture of the membranes can be used effectively to predict infection outcome in this group of patients. The nonstress test is noninvasive, simple, and a reasonably reliable surveillance tool that should be used in the clinical management of patients with premature rupture of the membranes to improve outcome.

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Limitations of using maximum vertical pocket and other sonographic evaluations of amniotic fluid volume to predict fetal growth: Technical or physiologic?

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Maximum vertical pocket measurement and subjective sonographic impression of amniotic fluid volume were studied in 487 selected pregnancies. Opiate abuse, suspected fetal growth retardation, and suspected postterm gestation were negatively correlated with maximum vertical pocket; suspected fetal growth acceleration and increasing birth weight were positively correlated. Maximum vertical pocket appeared to peak at 31 to 33 weeks ($p < 0.05$), and was related to fetal growth ($p < 0.001$). Subjective sonographic evaluation of amniotic fluid volume yielded comparable results. Unfortunately, neither technique could be used to accurately predict the birth of a small, appropriate, or large for gestational age infant in the individual pregnancy. Abnormal fetal growth could be reliably excluded in only a small number of cases. This and previous studies suggest that diagnosis of abnormal fetal growth by means of sonographic amniotic fluid volume evaluation could be limited by physiologic variation rather than technical error in measurement. (AM J OBSTET GYNECOL 1986;155:154-8.)

Key words: Maximum vertical pocket, amniotic fluid volume, ultrasound, fetal growth, intrauterine growth retardation

Clinical diagnoses of oligohydramnios and polyhydramnios, although hampered by imprecise criteria, have been associated with intrauterine growth retardation (IUGR), postmaturity, macrosomia, and fetal distress during labor.¹⁻⁴ Sonographic evaluation of amniotic fluid volume has been used in several attempts to improve diagnostic criteria and to predict these fetal problems. Attempts have been based on quantitative (total intrauterine volume),⁵ qualitative (maximum amniotic fluid pocket),^{3, 6-10} and subjective¹¹ evaluation of amniotic fluid volume.

Reports of total intrauterine volume determination as a predictor for IUGR have been inconsistent.^{5, 12} This measurement also has the practical limitation of requiring static imaging, which is unavailable in many obstetric ultrasound units. Real-time imaging has facilitated qualitative and subjective methods of assessing amniotic fluid volume and has served as the basis for several numerical definitions. Proposed criteria for oligohydramnios have included maximum pockets of < 1 or 2 cm in two planes.^{3, 7} Similarly, polyhydramnios has

been defined as a maximum pocket of 2 cm by 8 cm or more.⁸ However, the value of these criteria in the diagnosis of abnormal fetal growth has been limited.^{10, 13}

Recently, classifications based on the maximum vertical pocket of amniotic fluid in a single plane have been introduced.^{6, 11} This approach has the advantage of easy application in a busy clinical setting. A preliminary report suggests that this measurement is independent of gestational age and that it appears to be useful for the diagnosis of IUGR.⁶

The purpose of this study was to provide a comprehensive appraisal of the value of the maximum vertical pocket in detecting abnormal fetal growth. Specific aims included identification of major determinants of maximum vertical pocket, detailed analysis of the relationships of maximum vertical pocket with gestational age and fetal growth, evaluation of the performance of maximum vertical pocket as a diagnostic test for abnormal fetal growth, comparison of maximum vertical pocket with subjective sonographic evaluation of amniotic fluid volume, and interpretation of results in the perspective of previous studies that used different techniques.

Material and methods

This study included 487 pregnancies with intact membranes at the time of ultrasound examination performed between 26 and 43 weeks of gestation with delivery at our hospital between October and December

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Table I. Clinical correlations with maximum vertical pocket of amniotic fluid

Clinical factor	Frequency or mean $\pm$ 1 SD	r
Maternal weight (pounds, mean $\pm$ 1 SD)	164 $\pm$ 39	0.07
Poor maternal weight gain (%)	3	-0.07
Chronic hypertension (%)	4	0.07
Pregnancy-induced hypertension (%)	4	0.01
Diabetes mellitus (%)	7	0.04
Cigarette smoking	10	0.00
Opiate abuse (%)	10	-0.15*
Suspected small for gestational age (%)	24	-0.11†
Suspected large for gestational age (%)	16	0.17*
Suspected postterm (>42 wk) (%)	8	-0.13*
Birth weight (gm, mean $\pm$ 1 SD)	3120 $\pm$ 630	0.14*
Gestational age at birth (wk, mean $\pm$ 1 SD)	39 $\pm$ 2	-0.07

*p < 0.01.

†p < 0.05.

of 1984. Ultrasound examinations performed after rupture of the membranes, multiple gestations, stillbirths, and pregnancies with incomplete ultrasound results and/or clinical records were excluded. Gestational age at the time of ultrasound examination was calculated with the results of routine pediatric assessment (Ballard score). Classification of pregnancies as small, appropriate, or large for gestational age was based on the tenth and ninetieth percentiles of birth weight for gestational age at sea level. The study sample was composed of 65 (13%) small for gestational age, 366 (75%) appropriate for gestational age, and 56 (11%) large for gestational age pregnancies.

Indications for ultrasound examination included pregnancy dating, medical/obstetric problems (for instance, hypertension and diabetes), and amniocentesis. Only level II obstetric ultrasound examinations were performed in our laboratory by three certified sonographers under faculty supervision, using a real-time system (Technicare linear/sector Model 280, 3.5 mHz). Fetal morphometric measurements, placental grade, subjective evaluation of amniotic fluid volume, and maximum vertical pocket were obtained routinely. Subjective evaluation of amniotic fluid volume was classified by the sonographer as oligohydramnios, decreased fluid, normal fluid, increased fluid, or polyhydramnios. The maximum vertical pocket was measured by electronic calipers with the transducer perpendicular to the sagittal plane of the maternal abdomen, immediately over the point where the amniotic fluid volume appeared greatest.

Only the results of the last ultrasound examination

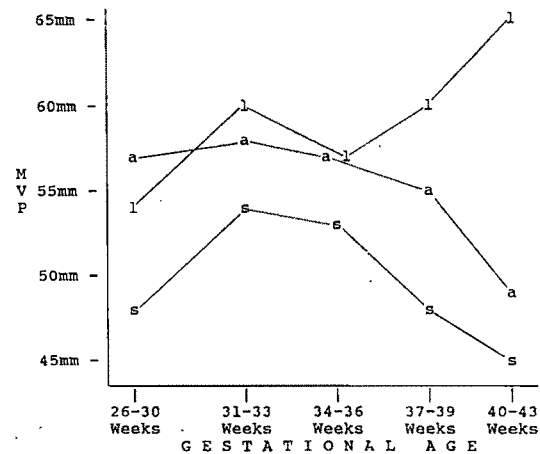


Fig. 1. Maximum vertical pocket (MVP) of amniotic fluid by gestational age and growth. Mean values for small (s), appropriate (a), and large (l) for gestational age infants are indicated.

were used for analysis. Simple correlation and analysis of variance, with the Brown-Forsythe statistic to adjust for inequality of variance where appropriate, were used to analyze determinants of maximum vertical pocket. Receiver operating characteristic curves were plotted to evaluate the performance of maximum vertical pocket as a diagnostic test for fetal growth abnormalities.¹⁴ Jackknifed discriminant function analysis was used to assess the effect of the duration between ultrasound examination and delivery on maximum vertical pocket as a diagnostic test. In order to adjust for the multiple comparisons made in this study, p < 0.05 was considered suggestive and p < 0.01 was considered significant.

Results

Pertinent clinical characteristics of the study sample and the correlations of these characteristics with maximum vertical pocket are reported in Table I. There were negative correlations with opiate abuse, suspected small for gestational age fetuses, and suspected post-term gestations. Maximum vertical pocket was positively correlated with suspected larger for gestational age fetuses and birth weight and appeared to be independent of gestational age. Despite statistical significance, none of these clinical factors accounted for more than 3% of the variance in maximum vertical pocket.

Detailed analysis of the relationships of maximum vertical pocket to gestational age and fetal growth is illustrated in Fig. 1 and detailed in Table II. Maximum vertical pocket was significantly related to growth (analysis of variance, p < 0.001). There was also a suggestion of an overall peak in maximum vertical pocket at 31 to 33 weeks (analysis of variance, p < 0.05). In contrast to the general pattern, maximum vertical pocket appeared to increase during later gestation among large

Table II. Maximum vertical pocket of amniotic fluid by gestational age and growth

	Maximum vertical pocket (mm)									
	26-30 wk		31-33 wk		34-36 wk		37-39 wk		40-43 wk	
	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n
Small for gestational age	48 $\pm$ 7	9	54 $\pm$ 11	12	53 $\pm$ 9	15	48 $\pm$ 18	14	45 $\pm$ 12	15
Appropriate for gestational age	57 $\pm$ 13	59	58 $\pm$ 14	68	57 $\pm$ 12	94	55 $\pm$ 13	91	49 $\pm$ 19	54
Large for gestational age	54 $\pm$ 12	9	60 $\pm$ 10	12	57 $\pm$ 16	10	60 $\pm$ 18	13	65 $\pm$ 20	12

Table III. Relationships of subjective evaluation of amniotic fluid volume by ultrasound to maximum vertical pocket of amniotic fluid and fetal growth

	Oligohydramnios	Decreased fluid	Normal fluid	Increased fluid	Polyhydramnios
N	2 (0.4%)	90 (18%)	379 (78%)	14 (3%)	2 (0.4%)
Maximum vertical pocket (mm) (mean $\pm$ SD)	24 $\pm$ 7	44 $\pm$ 14	57 $\pm$ 12	77 $\pm$ 16	86 $\pm$ 11
Cumulative small for gestational age					
N	0 (0%)	21 (32%)	65 (100%)	65 (100%)	65 (100%)
Sensitivity (%)	0	32	100	100	100
Specificity (%)	100	83	4	0	0
Cumulative large for gestational age					
N	0 (0%)	6 (11%)	51 (91%)	56 (100%)	56 (100%)
Sensitivity (%)	100	100	89	9	0
Specificity (%)	0	0	20	97	100

for gestational age pregnancies. In further analyses of variance (not illustrated), the values reported in Table II were not significantly affected by adjusting for the duration between ultrasound examination and delivery or by limiting analysis to cases having ultrasound examination <1 week before delivery.

Despite statistical significance, maximum vertical pocket alone performed poorly as a diagnostic test for abnormal fetal growth. It is apparent from Table II that mean values of maximum vertical pocket for small for gestational age, appropriate for gestational age, and large for gestational age varied in a range of only 20 mm and that standard deviations varied in a range just as large. The receiver operating characteristic curves for maximum vertical pocket as a diagnostic test for small for gestational age and large for gestational age are illustrated in Fig. 2 and Fig. 3, respectively. Both receiver operating characteristic curves are only slightly above the imaginary 45-degree line expected for a test with no diagnostic value. Maximum vertical pocket alone could not be used to reliably confirm small for gestational age or large for gestational age. Only 19% of cases with maximum vertical pocket <30 mm (3% of pregnancies) were small for gestational age; only 19% of cases with maximum vertical pocket >81 mm (3% of pregnancies) were large for gestational age. Extreme values of maximum vertical pocket could be used to exclude abnormal growth in a limited number of cases. No cases of small for gestational age had a maximum vertical pocket >80 mm (4% of pregnancies); no

cases of large for gestational age had a maximum vertical pocket <30 mm (3% of pregnancies). In discriminant function analyses (not illustrated), the performance of maximum vertical pocket as a diagnostic test was not significantly improved by adjusting for the duration between ultrasound examination and delivery or by limiting analysis to cases having ultrasound examination <1 week before delivery.

The relationships of subjective evaluation of amniotic fluid volume with the use of ultrasound to maximum vertical pocket and fetal growth are outlined in Table III. Amniotic fluid volume was rarely classified as abnormal (oligohydramnios or polyhydramnios) under the conditions of this study (viable singleton pregnancies with intact membranes at 26 to 43 weeks). A three-category classification (decreased, normal, or increased) would have yielded information regarding fetal growth equivalent to the five-category classification used in this study. For diagnosis of small for gestational age, oligohydramnios or decreased fluid (19% of pregnancies) had a sensitivity of 32% and a false positive rate of 17%, which was comparable to a sensitivity of 31% and a false positive rate of 18% for a maximum vertical pocket of 42 mm (19% of pregnancies). Similarly, for diagnosis of large for gestational age, increased fluid or polyhydramnios (3% of pregnancies) had a sensitivity of 9% and a false positive rate of 3%, which was comparable to a sensitivity of 9% and a false positive rate of 4% for a maximum vertical pocket of 80 mm (4% of pregnancies).

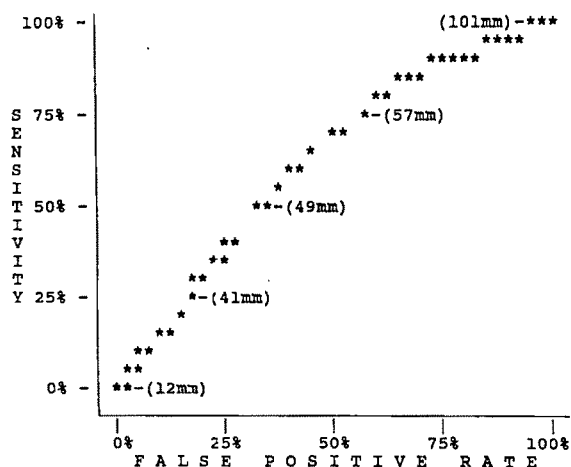


Fig. 2. Receiver operating characteristic curve for maximum vertical pocket of amniotic fluid in the diagnosis of small for gestational age pregnancy. Corresponding values for maximum vertical pocket are indicated in parentheses.

Comment

Preliminary reports of new tests to detect abnormal fetal growth have often been found to be overly optimistic when applied in routine clinical practice. Three explanations for these disappointments commonly noted in the obstetric literature are applicable to evaluation of maximum vertical pocket.

The first reason is that the apparent measures of merit of a diagnostic test (for instance, sensitivity and false positive rate) are partly dependent on the incidence of the diagnosis in the study sample.¹⁵ In the case of amniotic fluid volume evaluation, it has previously been noted that studies reporting high sensitivities for IUGR have been performed in subsets of patients selected to have high rates of IUGR based on clinical screening.^{10, 15} Since the frequencies of small for gestational age and large for gestational age in this study were only 13% and 11%, respectively, it might be anticipated that the measures of merit for maximum vertical pocket in diagnosing these conditions would be less than those reported in studies with more extensive preselection of patients. However, our results might also be expected to be more reproducible in routine clinical practice.

A second problem has been in choosing optimal rules for interpreting diagnostic tests. Receiver operating characteristic curves, as used in this study, can be helpful in avoiding this problem by facilitating analysis of the trade-offs between sensitivity and false positive rate.¹⁴ Although several rules for the diagnosis of abnormal fetal growth from sonographic amniotic fluid volume evaluation have been recommended, we are unaware of any that has been subjected to receiver operating characteristic curve or comparable analysis be-

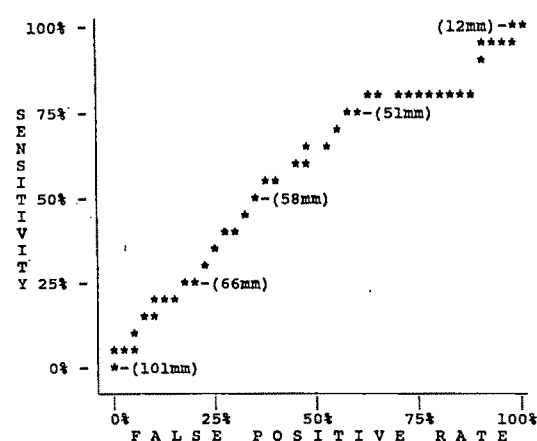


Fig. 3. Receiver operating characteristic curve for maximum vertical pocket of amniotic fluid in the diagnosis of large for gestational age pregnancy. Corresponding values for maximum vertical pocket are indicated in parentheses.

fore this study. This issue is particularly relevant, since it has been suggested that the low sensitivity of the "1 cm rule" might be increased by basing a rule on a larger pocket size (for instance, 2.0 cm).⁷

A third explanation is that diagnostic tests may have been performed with poor technique. Logically, variation in maximum vertical pocket can be attributed to two sources—technical and physiologic. In either case, our study would suggest that maximum vertical pocket alone would have little value as a diagnostic test in routine clinical practice. However, the possibility that physiologic variation is the primary limitation deserves further consideration. In this study, the diagnostic performance of maximum vertical pocket and subjective sonographic evaluation were remarkably similar and consistent with the findings of several previous studies based on quantitative, qualitative, or subjective methods of evaluating amniotic fluid volume.¹¹⁻¹³ It seems unlikely that this degree of similarity between different methods could be based on technical error alone. The observation that maximum vertical pocket is not entirely independent of gestational age, which may have been missed in previous studies because of use of only a straight-line model for analysis or lack of stratification for fetal growth, provides further evidence of physiologic variation.

In conclusion, it appears that maximum vertical pocket is comparable to subjective evaluation with the use of ultrasound. From the clinical perspective, amniotic fluid volume evaluation should alter the index of suspicion of fetal growth abnormalities (small for gestational age or large for gestational age), but physiologic variation appears to prohibit achieving diagnostic accuracy when used alone. From the research perspective, using amniotic fluid volume evaluation in combi-

nation with other parameters seems to be a more promising area for future studies than making further attempts to base rules solely on amniotic fluid volume evaluation.

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Coxsackievirus-positive cervixes in women with febrile illnesses during the third trimester in pregnancy

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Coxsackievirus B5 infection was demonstrated in five of seven third-trimester pregnant women with undifferentiated febrile illnesses or aseptic meningitis. Coxsackievirus B5 was recovered from the cervix and throat in four women and from the rectum in three. No obvious illnesses were evident in the babies. These findings suggest that previously unrecognized cervical enterovirus carriage or infection is common in infected pregnant women in the last trimester and that subsequent neonatal infection at delivery may result. (AM J OBSTET GYNECOL 1986;155:159-61.)

Key words: Coxsackievirus-positive cervixes, febrile pregnant women, enterovirus infection in pregnancy

Enterovirus infections are very common and in the neonate can lead to fatal myocarditis, encephalitis, or necrotizing hepatitis.^{1,2} However, little is known about the epidemiology of neonatal enterovirus infections. Transplacental viremia before delivery is possible, but the evidence for these occurrences is meager. Infection of the infant at the time of delivery by fecal or cervical contamination and postnatal contamination in the nursery may occur. Two recent case reports in which echovirus 11 was isolated from the cervix suggest contagion of the infant either during passage through a contaminated birth canal at delivery or by ascending infection from the cervix.^{3,4} This study was done to attempt to understand better the possible role of a virus-positive cervix in maternal third-trimester enterovirus infections.

Material and methods

From July through December of 1983, pregnant women in their third trimester who were ill on admission to St. John or Hutzel Hospitals were selected. These women had febrile illnesses ($\geq 38.3^{\circ}\text{C}$) without obvious other cause such as a urinary tract infection and were attending large, urban prenatal clinics at these institutions. They were suspected of having viral illnesses. Workup for the cause of fever included routine blood and urine cultures as well as cultures of other body fluids such as cerebrospinal fluid, when indicated.

Swabs from the cervix, rectum, and throat of these

ill gravid women were obtained for enterovirus culture and transported to the Infectious Disease Research Laboratory in 1 ml volumes of sterile minimal essential medium supplemented with penicillin G, 100 U/ml, and gentamicin, 100 $\mu\text{g}/\text{ml}$. One tenth milliliter of each specimen was promptly inoculated into two tube tissue cultures each of Vero African green monkey kidney, human rhabdomyosarcoma (ATCC, Rockville, Maryland) and primary human foreskin fibroblast cells. Inoculated and noninoculated control cultures were maintained in 1.5 ml of minimal essential medium containing 5% fetal bovine serum and incubated for 2 weeks at 37°C . Supernates from tube cultures showing cytopathic effects were harvested and frozen at -70°C in sterile polypropylene vials. Specimens were taken again at revisit from two patients. Acute and convalescent sera were obtained when possible. Careful clinical and virologic study was done at birth in one baby whose mother was delivered during the course of the febrile illness. This study protocol was approved by the Human Investigation Committee of Wayne State University. Patients enrolled into the study signed consent forms.

Viruses were identified by neutralization test with use of antiserum pools. These results were confirmed by neutralization with monospecific rabbit antisera (Telcolab Corporation, New York). Viral isolates were confirmed by the Microbiology Reference Laboratory in Cypress, California.

With use of microtiter wells of vero monkey kidney tissue cultures, sera from mothers were tested for IgM- and IgG-neutralizing antibodies to coxsackievirus types B1 to B5. IgM- and IgG-neutralizing antibodies were measured before and after treatments of sera with 2-mercaptoethanol and iodoacetamide. Antibody titers were reported as the highest serum dilution that com-

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Table I. Clinical illnesses and coxsackievirus B5 (CB5) isolations from cervixes of women in the third trimester of pregnancy

Patient	Age (yr)	Gestational age (wk)	Date of delivery	Mode of delivery	Viral illness	Virus isolations		
						Date	Cervix	Other
1	28	36	8/83	Vaginal	Undifferentiated febrile illness	7/6/83	+	Rectum +; throat +
						7/28/83	—	—
						8/14/83	—	—
2	25	30	9/11/83	Vaginal	Aseptic meningitis	8/8/83	+	Rectum +; throat +; CSF +
						8/17/83	—	—
						9/11/83	—	Amniotic fluid —
						10/4/83	—	—
3	29	31-32	12/7/83	Vaginal	Undifferentiated febrile illness	9/14/83	+	Rectum +; throat —
4	18	42	10/25/83	Cesarean section	Aseptic meningitis	10/24/83	+	Rectum —; throat +; CSF —
5	36	36	10/25/83	Repeat cesarean section	Undifferentiated febrile illness	10/10/83	—	Rectum —; throat +
						10/24/83	—	—

+ = Positive. — = Negative. CSF = Cerebrospinal fluid. ND = Not done.

pletely prevented the appearance of viral cytopathic effects. Details of these methods have been reported.⁴

Results

Seven febrile women between the thirtieth to forty-second week of pregnancy suspected to have viral illnesses were studied (Table I). They were 18 to 36 years old.

Cervical swabs from four of the seven women yielded coxsackievirus B5. Coxsackievirus B5 was also recovered from the throat (4x) or rectum (3x). Coxsackievirus B5 was isolated from the throat of one patient whose cervix and rectum were virus negative. In two women cultures of cervix, rectum, and throat were repeated 9 and 22 days later. They were negative.

Two of the seven mothers had IgM titers of 1:64 to coxsackievirus B5 (cases 1 and 3). Case 5 showed a rise in neutralizing IgG antibody titers to coxsackievirus B5. One baby, whose mother was delivered by cesarean section during the febrile illness and whose cervix was virus positive (Case 4), was culture negative at birth.

Comment

The prevalence of enteroviruses among pregnant women has been studied. When pregnant women at term were studied, Modlin et al.⁵ showed that the excretion rate was 3.6% during a 3-week period in the midst of a community outbreak of echovirus 11 infections. Six isolates were from the gastrointestinal tract, and the seventh was from the throat. Cherry et al.⁶ had earlier found a similar enterovirus isolation rate (4% in 55 women) in the last 6 weeks of pregnancy. En-

terovirus isolations were mainly from the gastrointestinal tract. Vaginal cultures were obtained, but no viruses were found. In 1984, within 1 day of delivery, throat and rectal swabs were taken from 629 women, but no enteroviruses were isolated.⁷ A 57% risk of perinatal transmission of echovirus 11 was noted in a small group of virus-positive mothers (four of seven), while the overall incidence of infection was only 2.5%.²

In this study, cultures from five of seven (71%) third-trimester pregnant women with febrile illnesses suspected to be of viral origin yielded enterovirus isolates, all of which were coxsackievirus B5. Cervical and throat swabs were most productive (four of five); rectal swabs were positive in three of four women. This high isolation rate is extraordinary and relates in part to a high index of suspicion for viral illness and collection of specimens during the acute illnesses. Most interesting is the repeated isolation of coxsackievirus B5 virus from the cervix in this small group of patients. It certainly suggests that cervical contamination or infection of the cervix is not unusual. The clinical significance of these findings remains to be determined. The four febrile mothers with cervical virus were not in labor during their acute coxsackievirus B5 infections. They were delivered from 1 to 84 days later, and the duration of cervical coxsackievirus B5 shedding is unknown. Except for transient cardiac arrhythmia in one fetus, however, transplacental infection or the possible morbid consequences of coxsackievirus B5 infection were not observed in these infants. We do know as well that afebrile pregnant women do not commonly harbor cervical enteroviruses.⁷ In an earlier study we were unable

Neutralizing antibody to CB5		Outcome	
IgM	IgG	Mother	Baby
		Well	Healthy
1:64	<1:16		
1:16	<1:16		
<1/8	<1:16	Well	Fetal heart irregularities; healthy at delivery
ND	ND		
ND	ND		
<1:8	<1:16		
1:64	1:16	Well	Healthy
<1:8	<1:16	Well	Healthy; culture negative for CB5
1:8	1:16	Well	Healthy
1:16	1:128		

to detect enteroviruses in the cervix, throat, or rectum of 71 asymptomatic women including 19 nonpregnant, 11 first-trimester, 20 second-trimester, and 21 third-trimester patients (unpublished observations).

Coxsackievirus B5 was the most prevalent enterovirus in the United States in 1983.⁸ Indeed, the isolation of coxsackievirus B5 from our patients reflects its similar occurrence in Michigan that year. Coxsackievirus B5 may cause serious generalized disease in the newborn infant. Along with group B coxsackievirus serotypes 1 to 4, coxsackievirus B5 is most frequently associated with myocarditis and, frequently, encephalitis in the newborn infant.^{9,10} Outbreaks of coxsackievirus

B5 infections in nurseries also occur.¹¹ Further information as to their spread could also decrease morbidity. The frequent isolations of coxsackievirus B5 from cervixes of febrile women in the third trimester of pregnancy reported here raise the possibility of neonatal enterovirus infection at delivery from a contaminated cervix, a possibility that seems apparent here but has not previously been seriously considered. Studies of the possible importance of these findings certainly seems warranted.

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Vasopressor activity in incubated plasma of normal and hypertensive pregnant women

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The pressor activity generated in incubated plasma of nonpregnant, normal pregnant, and hypertensive pregnant women was measured by means of a sensitive bioassay technique. It was found that the plasma of normal pregnant women generated significantly higher amounts of active pressor principle than the plasma of nonpregnant women. The plasma of hypertensive pregnant women generated significantly lower amounts of active pressor principle than the plasma of normal pregnant women. Plasma obtained from the antecubital vein revealed no difference in pressor activity compared to plasma collected from the uterine vein at the time of cesarean section. These data suggest that active pressor principle is not involved in the pathogenesis of pregnancy-induced hypertension and that the pregnant uterus is not the source of active pressor principle. (AM J OBSTET GYNECOL 1986;155:162-5.)

Key words: Vasopressor substance, plasma, human, pregnancy-induced hypertension

Incubation of human plasma at 37° C for several hours leads to the formation of a nondialyzable vasopressor substance, termed the active pressor principle.¹⁻³ We previously demonstrated that active pressor principle is a heat-stable peptide distinct from renin or angiotensins and that is formed by the action of enzymes that can be inhibited by diisopropyl fluorophosphate. Active pressor principle was crudely purified in alcoholic trichloroacetic acid solution after placing the plasma in a boiling water bath for 5 minutes. The molecular weight of active pressor principle as determined by gel filtration of this fraction was about 68,000.¹ However, the physiologic role of active pressor principle remains unknown.

The etiology of pregnancy-induced hypertension has long been the subject of extensive research and speculation. Attempts have been made to identify the pressor substance that causes pregnancy-induced hypertension, but none were successful.^{4,5} Increased formation of active pressor principle compared with the sera of nonpregnant women was found in incubated sera of pregnant women.⁶ We therefore decided to investigate, with use of a sensitive bioassay technique, whether or not patients with pregnancy-induced hypertension are characterized by increased active pressor principle.

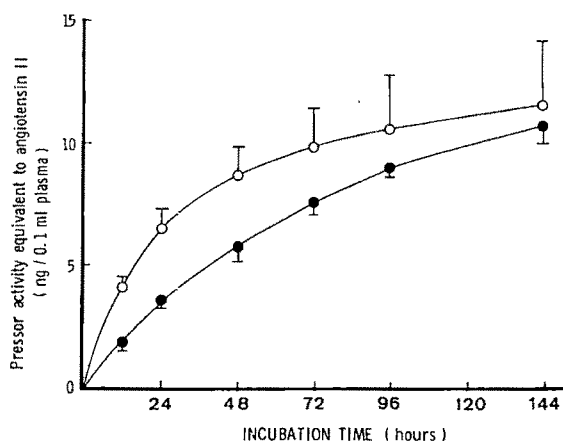


Fig. 1. Comparison of the time courses of active pressor principle formation in incubated plasma from nonpregnant women (closed circles) and from normal third-trimester pregnant women (open circles).

Material and methods

Five nonpregnant healthy women and five normal third-trimester pregnant women participated in the first study. Blood was withdrawn in a sterile manner and immediately centrifuged in a heparinized tube at 4° C (10 minutes at 2,000 × g). The plasma was then incubated for 12 to 144 hours at 37° C in an incubator and stored at -20° C until assay.

In a second series, blood was taken from 17 nonpregnant healthy women, 51 normal pregnant women, and 17 patients with pregnancy-induced hypertension. In the nonpregnant women the phase of the menstrual cycle was not taken into consideration at the time of blood collection, since it did not demonstrate any

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Table I. Clinical characteristics of nonpregnant, normal pregnant, and hypertensive pregnant women whose plasma was incubated for 48 hours and used for active pressor principle assay

	No. of cases	No. of primiparas	Age (yr)	Gestational time (wk)	
				Range	Mean $\pm$ SD
Nonpregnant	17	14*	26.52 $\pm$ 3.18		
1st trimester, normotensive	17	12	29.08 $\pm$ 3.80	7-15	9.59 $\pm$ 2.48
2nd trimester, normotensive	17	12	27.88 $\pm$ 3.22	16-27	20.82 $\pm$ 2.74
3rd trimester, normotensive	17	12	28.33 $\pm$ 3.92	28-41	35.64 $\pm$ 2.87
3rd trimester, hypertensive	17	12	28.43 $\pm$ 5.68	28-41	35.47 $\pm$ 2.50

Values are means $\pm$ SD.

*No. of nulliparas.

significant effect on active pressor principle formation. The normal pregnant women were divided into three groups: first trimester ($n = 17$), second trimester ($n = 17$), and third trimester ($n = 17$) of pregnancy. All women remained normotensive throughout their pregnancy. The women with pregnancy-induced hypertension were known to be normotensive early in pregnancy and post partum. None of the subjects had a history of metabolic, cardiovascular, or renal disease. During the last 12 weeks of pregnancy each of the subjects revealed an increase in diastolic blood pressure of at least 20 mm Hg and a blood pressure of no less than 140/90 mm Hg. Sixteen of the patients with pregnancy-induced hypertension had proteinuria of no less than 300 mg/24 hours. Patients who had overt clotting problems, oliguria, pulmonary edema, persistent epigastric pain, or elevation of liver transaminases were not included in this study. None had taken diuretics or antihypertensive drugs. Age and parity were matched in all groups. Normal third-trimester pregnant women and patients with pregnancy-induced hypertension were also matched for gestational weeks. The plasma samples were incubated for 48 hours at 37° C and stored at -20° C until assay.

In a third series, blood was withdrawn from the uterine vein as well as from the antecubital vein simultaneously at the time of cesarean section in seven term pregnant women. The mean gestational age of the seven patients was 38.8 weeks (range, 37 to 40). Four patients were primigravidas. The indications for cesarean section were cephalopelvic disproportion ($n = 2$), intrauterine growth retardation ($n = 2$), breech presentation ($n = 2$), and a history of uterine incision ($n = 1$). Epidural anesthesia was carried out in four patients and spinal anesthesia in three patients. Blood was obtained from the uterine vein just before the uterine incision and from the antecubital vein at the same time. The plasma was incubated for 12 to 72 hours at

37° C and stored at -20° C until assay. Patients with pregnancy-induced hypertension were not included in this study.

Informed consent was obtained from the subjects for their blood collection. Incubated plasma samples were confirmed several times to be free from aerobic/anaerobic bacteria or fungi by culture in trypticase soy agar.

Assay method of active pressor principle. The previously described assay method for active pressor principle was used in these studies.¹ Briefly, male white Wistar rats (weighing 250 to 300 gm) were anesthetized by intraperitoneal injections of pentobarbital. Pentolinium (5 mg/rat) was administered subcutaneously. The trachea was cannulated. The right jugular vein and left carotid artery were cannulated with catheters. The arterial cannula was connected to a transducer, and the blood pressure, mean arterial pressure, and pulse rate were continuously recorded. Plasma samples to be assayed were injected intermittently as a bolus through the venous cannula every 8 to 10 minutes. The relative pressor response was assessed as described previously, that is, the amplitude of the rise in mean arterial pressure caused by each of the test samples was compared with that caused by 5 ng of angiotensin II, which was employed as a standard, and the pressor activity of the plasma samples was expressed as equivalent nanograms of angiotensin II. Each plasma sample was injected in a volume of ≤ 0.1 ml as a bolus. Each sample was tested in three rats. Control angiotensin II (5 ng in 0.1 ml of saline solution) was injected several times during each experiment to ensure constant sensitivity for each rat assay preparation. All samples were assayed within 2 months. After storage of incubated plasma at -20° C for 2 months, no change in plasma vasopressor activity was shown. The results were expressed as mean $\pm$ standard error of the mean, and the means were compared by the paired and unpaired Student's *t* test.

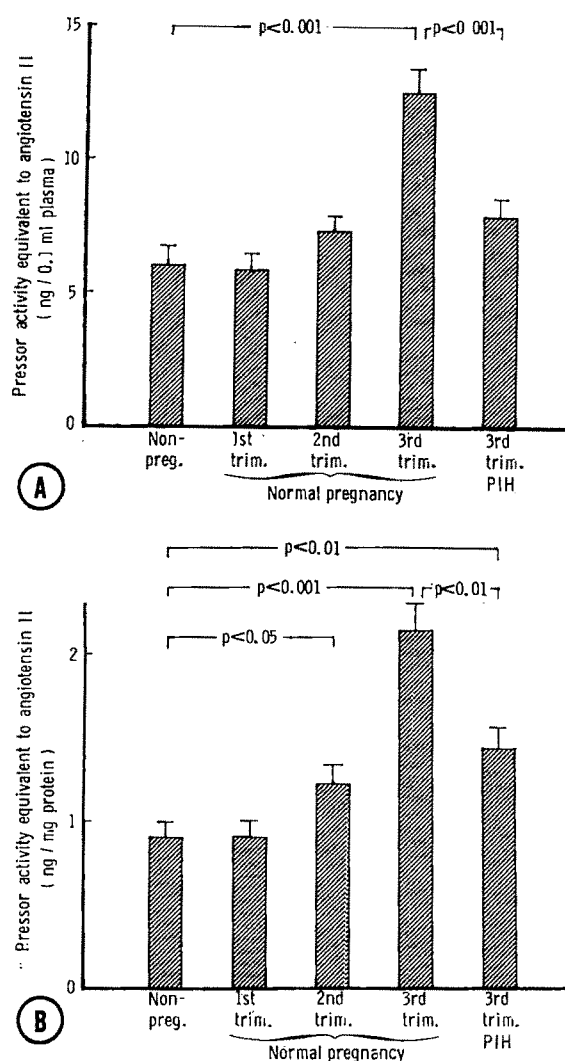


Fig. 2. Pressor activity of incubated plasma from nonpregnant women, normal pregnant women (first, second, and third trimesters), and hypertensive pregnant women after incubation of the plasma for 48 hours at 37° C. The pressor activity is expressed in equivalent nanograms of angiotensin II per 0.1 ml of plasma (A) or per 1 mg of protein (B).

Results

Incubation of plasma from nonpregnant and normal third-trimester pregnant women. Incubation of plasma obtained from normal pregnant women in the third trimester revealed a gradual increase in pressor activity, which was more abrupt than that of plasma from nonpregnant women (Fig. 1). The pressor activity was significantly higher in the pregnant women at incubation periods of 12, 24, and 48 hours ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively).

Incubation of plasma from nonpregnant, normal pregnant and hypertensive pregnant women. Some of the clinical features of each group studied are presented in Table I. Since the biochemical characterization of active pressor principle had been previously

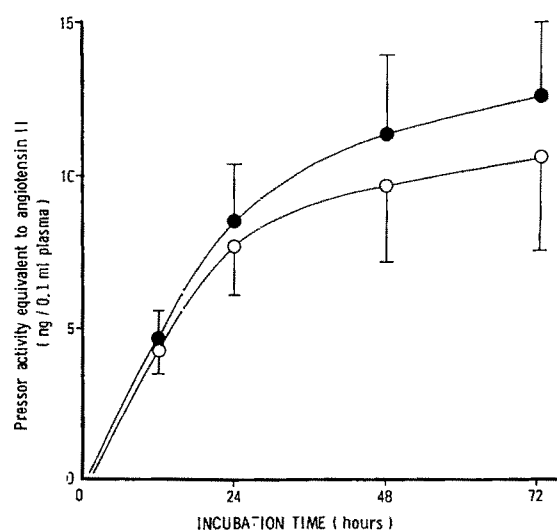


Fig. 3. Comparison of the time courses of active pressor principle formation in plasma obtained from the antecubital vein (closed circles) and plasma obtained from the uterine vein (open circles) at the time of cesarean section.

carried out with use of 48-hour incubated plasma,¹ the active pressor principle levels in this series were assessed with 48-hour incubated plasma. The active pressor principle levels for the nonpregnant, normal pregnant, and hypertensive pregnant women are shown in Fig. 2, where the pressor activity is expressed in equivalent nanograms of angiotensin II per 0.1 ml of plasma or per 1 mg of protein. The active pressor principle levels in the nonpregnant, first-trimester, second-trimester, third-trimester (normotensive), and third-trimester (hypertensive) pregnant women were 6.10 ± 0.63 , 5.86 ± 0.58 , 7.30 ± 0.57 , 12.56 ± 1.00 , and 7.80 ± 0.77 equivalent nanograms of angiotensin II per 0.1 ml of plasma, respectively, and 0.90 ± 0.09 , 0.90 ± 0.10 , 1.22 ± 0.11 , 2.14 ± 0.16 , and 1.43 ± 0.15 equivalent nanograms of angiotensin II per 1 mg of protein, respectively. For both sets of figures, the active pressor principle levels in the normal third-trimester pregnant women were significantly higher than those in the nonpregnant women, and the active pressor principle levels in the hypertensive pregnant women were significantly lower than those in the normal third-trimester pregnant women.

Incubation of plasma from the uterine vein and peripheral vein. Plasma obtained from the uterine vein revealed an increase in pressor activity during incubation. However, the pressor activity at each incubation period was not statistically different from that obtained from the antecubital vein (Fig. 3).

Comment

The active pressor principle levels of incubated plasma were found to be higher as pregnancy pro-

ceeded, and the levels in third-trimester pregnant women indicated a significantly higher pressor activity than that in nonpregnant women. Our results are in accord with those reported by Hertlein et al.⁶ and are also consistent with those reported by Katz et al.,³ who demonstrated increased active pressor principle levels in incubated plasma of pregnant rabbits. It is of interest to note that blood pressure normally decreases in early gestation but returns toward nonpregnant values in late gestation. Increased active pressor principle levels in late gestation may, at least in part, be responsible for these changes in blood pressure.

However, the pressor activity of the plasma during hypertensive pregnancy is an important issue. The active pressor principle levels in the patients with pregnancy-induced hypertension were significantly lower than those in normal pregnant women. The results suggest that active pressor principle may not be the vasopressor substance which causes pregnancy-induced hypertension, but it could be involved in blood pressure regulation as a compensatory mechanism. Active pressor principle is considered to be a physiologic antagonist in blood pressure regulation. Pregnancy-induced hypertension is characterized by vasospasm⁷ in spite of the lowered active pressor principle levels. Although the pathophysiology of pregnancy-induced hypertension could not be attributed to increased active pressor principle levels, it is possible that patients with pregnancy-induced hypertension may develop altered vascular sensitivity to circulating active pressor principle, which could induce pregnancy-induced hypertension.

The source of the increased active pressor principle levels during pregnancy is not yet known. Attempts

were made to demonstrate fetoplacental production of active pressor principle. However, the active pressor principle levels in the uterine vein were not significantly different from those in the peripheral vein or were slightly lower. This means that fetoplacental production of active pressor principle could not be demonstrated in the present study.

Further investigations on the active pressor principle levels in various pathologic states are now in progress.

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Hematologic evidence of fetal hypoxia among newborn infants at high altitude in Bolivia

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The reduced birth weight widely reported for high-altitude infants has been attributed to intrauterine hypoxia. This assertion may be tested by comparing hematologic values of high-altitude and low-altitude newborn infants, since the human fetus can respond to intrauterine hypoxia with enhanced erythropoiesis. This report presents cord blood values of term Bolivian infants classified by altitude, ethnic group, and parity: 105 from La Paz (3600 m) and 46 from Santa Cruz (400 m). Hematocrit, hemoglobin concentration, and the proportion of hemoglobin F are higher among newborn infants at high altitude. Ethnic group and parity interact with altitude in effect on hematocrit and hemoglobin concentration. Gestational age, birth weight, maternal hematologic variables, and socioeconomic indicators are not significantly associated with cord blood values. The high-altitude newborn infant displays evidence of enhanced erythropoiesis, which supports the suggestion that the high-altitude fetus experiences a greater degree of hypoxia in utero than does the low-altitude fetus. (AM J OBSTET GYNECOL 1986;155:166-9.)

Key words: Fetal hypoxia, high altitude, hematology

In the past 25 years there has been ample confirmation of the observation that newborn infants at high altitude are smaller than their low-altitude counterparts.¹ This observation is often interpreted as evidence of fetal growth retardation resulting from hypoxia. However, this interpretation is based on a process of elimination: research strategies have generally controlled for other factors known to contribute to variation in birth weight, with the residual variation by altitude being attributed to hypoxia. Cell culture experiments and studies of gestation under hypoxic conditions in a number of animal species support this contention.¹ None of these is a completely satisfactory model for human pregnancy and fetal growth at high altitude, and it is difficult to directly assess fetal hypoxia in human pregnancy.

Among the indirect techniques that can be used to address the issue of fetal hypoxia at high altitude is a comparison of hematologic values of high-altitude and low-altitude newborn infants. After birth, high-altitude hypoxia is associated with enhanced erythropoiesis and consequent increases in hemoglobin concentration and hematocrit.² The human fetus is capable of producing erythropoietin by at least the eleventh week of gestation

and responds to intrauterine hypoxia with increases in hemoglobin concentration and hematocrit.³ Erythropoietin titers, hemoglobin concentration, and hematocrit in cord blood at birth are elevated in a variety of obstetric conditions which are anoxicogenic for the fetus, including preeclampsia, maternal cardiovascular disease, and smoking during pregnancy; the proportion of fetal hemoglobin, hemoglobin F, in the cord blood is also elevated in these conditions.³

If the human fetus at high altitude experiences a greater degree of hypoxic stress in utero than the fetus at low-altitude, the high-altitude newborn might display evidence of enhanced erythropoiesis or persistence of hemoglobin F at birth. The purpose of this study is to test this hypothesis in samples of newborn infants from high and low altitudes in Bolivia.

Methods

The hypothesis was tested in a Bolivian sample including 105 newborn infants from La Paz, at a mean altitude of 3600 m above sea level, and 46 newborn infants from Santa Cruz, at approximately 400 m above sea level. These infants and their mothers are part of an ongoing study of pregnancy and fetal and infant growth conducted by investigators from Cornell University. Sampling procedures and detailed descriptions of the sample have been published elsewhere.⁴ The sample includes women from lower through upper socioeconomic status; because participants were recruited from hospital and clinic patients, the sample excludes women of very low socioeconomic status. Two indices of socioeconomic status, monthly household income

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and years of mother's education, were included as independent variables in the analysis.

The infants that constitute this sample were delivered in hospitals to women who had had at least one prenatal examination and were experiencing normal pregnancies. Infants were classified as Indian or European on the basis of their mother's first language and surname. They were further classified as infants of primiparous or of multiparous women. Sex of the infant had no significant effect on the cord blood values and has been omitted from the analysis.

Only term infants were included in this sample, that is, infants of 260 days of gestation or more. Gestational age was estimated by the Dubowitz protocol with use of a regression equation derived specifically for this population.⁴ The mean gestational age of the infants in the sample was 274.7 days, with a standard deviation of 4.5 days. Gestational age did not vary significantly by altitude, ethnic group, or parity when tested by analysis of variance.

Infants were not selected on the basis of birth weight. In particular, infants weighing <2500 gm at birth were not excluded from the sample. Mean birth weights of the infants are presented in Table I. Analysis of variance indicates that infants at low altitude were heavier than infants at high altitude, Indian infants were heavier than European infants, and infants of multiparous women were heavier than infants of primiparous women. In this regard the infants included in this sample are similar to a larger sample of Bolivian newborn infants examined in La Paz and Santa Cruz⁴ and conform to the pattern also observed in the United States and Peru.¹

Heparinized cord blood was collected at delivery. Hematocrit was determined by the microcapillary method and total hemoglobin concentration by the cyanomet-hemoglobin method.⁵ The proportion of hemoglobin F was determined by alkali denaturation.⁶ All analyses were performed on whole blood within 4 hours of collection at the Instituto Nacional de Laboratorios de Salud in La Paz or in Cornell laboratory facilities in Santa Cruz.

Variation in cord blood values was assessed by simultaneous analysis of variance with use of the Statistical Package for the Social Sciences subprogram analysis of variance.⁷ This approach evaluates the contribution of each independent variable to variation in the dependent variable while adjusting simultaneously for all other significant independent variables. This requires no a priori assumptions about the relative importance of independent variables or about causal sequences. It is also the most suitable approach when opportunity sampling produces unbalanced cell sizes. Potential contributions of gestational age, maternal he-

Table I. Mean birth weight of newborn infants classified by altitude, ethnic group, and parity

	<i>N</i>	<i>Weight (gm)</i>	<i>SD</i>
All	151	3205	451
High altitude	105	3075	381
Indian	49	3134	365
Primiparous	21	2986	318
Multiparous	28	3245	363
European	56	3023	391
Primiparous	22	3048	386
Multiparous	34	3007	399
Low altitude	46	3500	462
Indian	19	3619	385
Primiparous	10	3619	436
Multiparous	9	3619	345
European	27	3416	500
Primiparous	8	3145	431
Multiparous	19	3530	492

On the basis of three-way analysis of variance, the following main effects are significant: altitude, $p < 0.001$; ethnic group, $p < 0.05$; parity, $p < 0.05$.

matologic variables, and socioeconomic indices to variation in cord blood values were assessed by analysis of covariance and partial correlation analysis, controlling for altitude.

Results

The mean values for cord blood hematocrit, total hemoglobin concentration, and the proportion of hemoglobin F are presented in Table II. The values for all three cord blood variables were higher among newborn infants at high altitude than among those at low altitude. The elevation of cord blood hematocrit at high altitude was greater among infants of primiparous women than among infants of multiparous women; this is indicated by the significant two-way interaction between altitude and parity. The elevation of cord blood total hemoglobin concentration at high altitude was greater among infants of primiparous women, and also greater among infants of European women than among infants of Indian women.

Several models, both additive and interactive, were tested to examine the relationship of cord blood values to birth weight and gestational age. Neither was significantly associated with variation in cord blood values in any of the models. In addition, there were no significant partial correlations between cord blood values and maternal hematologic values or socioeconomic indicators, controlling for altitude.

Comment

Previous investigators have found a negative correlation between gestational age and the proportion of hemoglobin F in cord blood, especially among preterm

Table II. Mean cord blood values of newborn infants classified by altitude, ethnic group, and parity

	<i>Hematocrit*</i> (vol%)			<i>Hemoglobin†</i> (gm/dl)			<i>Hemoglobin F‡</i> (%)		
	<i>N§</i>	<i>Mean</i>	<i>SD</i>	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>N</i>	<i>Mean</i>	<i>SD</i>
All	140	52.5	6.4	136	16.6	1.9	151	62.0	13.7
High altitude	92	53.5	6.4	89	17.1	1.8	105	64.4	13.3
Indian	45	52.4	6.6	44	16.6	1.7	49	63.8	13.8
Primiparous	20	54.2	7.4	19	16.6	1.5	21	58.9	15.6
Multiparous	25	51.0	5.7	25	16.6	1.8	28	67.5	11.2
European	47	54.6	6.1	45	17.6	1.8	56	65.0	13.0
Primiparous	20	56.6	6.6	19	17.8	1.6	22	64.8	12.1
Multiparous	27	53.2	5.3	26	17.3	1.9	34	65.1	13.8
Low altitude	48	50.6	6.1	47	15.5	1.8	46	56.5	13.1
Indian	18	51.4	5.7	18	15.8	1.8	19	55.0	11.2
Primiparous	11	50.0	4.7	10	15.4	1.5	10	56.7	9.1
Multiparous	7	53.7	6.7	8	16.4	2.1	9	53.2	13.5
European	30	50.1	6.4	29	15.3	1.8	27	57.5	14.4
Primiparous	10	50.4	7.1	10	14.5	1.5	8	59.5	9.0
Multiparous	20	49.9	6.2	19	15.8	1.8	19	56.6	16.3

*On the basis of three-way analysis of variance, the main effect of altitude is significant at $p < 0.05$.

†The main effect of altitude is significant at $p < 0.001$. Two-way interactions between altitude and parity ($p < 0.05$) and between altitude and ethnic group ($p < 0.005$) are also significant.

‡The main effect of altitude is significant at $p < 0.005$.

§Cell sizes vary slightly because of missing data. Cases have been included in all calculations for which data are available.

infants.³ Since our sample is of restricted gestational age, including only term infants, no such association is apparent in this analysis. High levels of hemoglobin F have also been reported among infants who are small for gestational age; intrauterine hypoxia is often implicated in the etiology of growth retardation in these infants.³ No association between any of the cord blood values and birth weight was found in this sample.

We included all normal term newborn infants in a single sample for the analysis. All were considered appropriate for gestational age by the attending obstetrician or pediatrician, and none required special treatment. No significant hematologic differences were found between infants weighing ≤ 2500 gm and infants weighing > 2500 gm, controlling for altitude.

The elevated levels of cord blood hematocrit, total hemoglobin concentration, and the proportion of hemoglobin F among newborn infants at high altitude, compared to those at low altitude, supports the assertion that the former experience a greater degree of hypoxic stress in utero. This interpretation may be complicated, however, by potential altitude differences in maternal and fetal iron status in the sample.

Direct measurements of neonatal iron status were not available for this analysis. Evaluation of the iron status of the women whose infants were included in our sample is not yet complete, but Haas⁸ found that many of the women had low or deficient iron stores in the last trimester of pregnancy, reflected in transferrin saturations of $< 15\%$. All of the women in the sample nonetheless had hemoglobin levels that were clinically acceptable according to norms for high-altitude gravid

women. Haas (unpublished data) further indicates that iron deficiency may be somewhat more prevalent among low-altitude gravid women than among gravid women of comparable ethnic and socioeconomic background at high altitude. Maternal iron deficiency anemia might contribute to fetal hypoxia at low altitude, hypobaric hypoxia notwithstanding. However, it is possible that maternal iron deficiency leads to fetal iron deficiency and limits the fetus' hematopoiesis. In this regard the fetus at high altitude may be able to respond more readily to intrauterine hypoxia than the fetus at low altitude.

The proportion of hemoglobin F in cord blood should be less affected than hematocrit or total hemoglobin concentration by maternal or fetal iron nutrition, since hemoglobin F and hemoglobin A contain the same amount of iron.³ The proportion of hemoglobin F in cord blood is, in fact, independent of maternal hematologic values except in cases of severe maternal anemia.⁹ Hemoglobin F may therefore be the most sensitive indicator of fetal hypoxia available in this study.

Elevated levels of hemoglobin F in cord blood are attributed to delayed switching from cell populations committed to the production of hemoglobin F to those committed primarily to the production of hemoglobin A.¹⁰ Hypoxia delays this switching in erythropoietic tissues in vitro.¹¹ Elevated levels of hemoglobin F in cord blood are reported among newborns who have experienced intrauterine hypoxia secondary to obstetric complications.³

The alkali denaturation method used in this inves-

tigation underestimates the true proportion of hemoglobin F, particularly at high levels.¹² It may, in addition, overestimate hemoglobin F at low levels, while the chromatographic method tends to overestimate hemoglobin F throughout the normal range.¹³ It is impossible to assess the effect of such a potential bias on the altitude contrast in the proportion of hemoglobin F in cord blood observed in this sample without a comparable series of chromatographic determinations, but technical considerations suggest that the true altitude difference in hemoglobin F may be even greater than that reflected in these results.

Previous hematologic studies of newborn infants at high altitude have generally not found significant increases in neonatal hematocrit or total hemoglobin concentration.¹ Those studies did not control for the ethnic group or parity of the subjects nor apparently for nutritional status. Clearly the iron status of the mother and fetus is an important variable which should be examined in future investigations.

In summary, the hematocrit, total hemoglobin concentration, and the proportion of hemoglobin F in cord blood among newborn infants at high altitude in Bolivia were higher than the values observed in newborn infants at low altitude. This suggests that the fetus at high altitudes experiences a greater degree of hypoxic stress than the fetus at low altitude. It is possible that this fetal hypoxia is due in part to inadequate maternal adjustments to hypoxia as a result of subclinical iron deficiency anemia. This suggestion is the focus of investigations currently in progress.

In view of previously reported evidence of differential iron status among pregnant women at high and low altitudes in Bolivia, the elevated hematocrit and total hemoglobin concentration among their newborn infants provide a limited estimate of hypoxia-induced erythropoiesis in utero. The proportion of hemoglobin F in cord blood may be the more useful index of fetal hypoxia in this context, since it is relatively independent of iron status.

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Functional development of human eye movement in utero assessed quantitatively with real-time ultrasound

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Functional development of eye movement in the human fetus in utero was assessed quantitatively, with real-time ultrasound. A pair of lens-derived echoes was used as a landmark to evaluate positional deviation with eye movement. The frequency of eye movement was measured either with 1-minute continuous observations or at 1-minute cross sections. Two hundred forty-five normal fetuses between 18 and 42 weeks of gestation were studied. Three critical ages, with respect to physiologic development of fetal eye movements, were evident: 22 to 25, 30 to 33, and 38 to 41 weeks. The first and the second indicate the onset of the moderate-frequency and high-frequency eye movements, thereby implying a transition, presumably to a state of so-called rapid eye movement and a rise of rapid eye movement, respectively. The third represents full maturation of the mechanisms, that is, non-rapid eye movement and rapid eye movement. (AM J OBSTET GYNECOL 1986;155:170-4.)

Key words: Eye movement, human fetus, functional development, real-time ultrasound, sleep regulation

Real-time ultrasound has facilitated observation of the functional aspects of fetal life, since it provides real-time images of any given fetal organ, which change either in shape or in position.

Special attention has been directed to the evaluation of fetal eye movement, because this information should provide a clue toward assessment of developmental processes related to sleep and wakefulness, in the human fetus in utero.

Bots et al.¹ showed that actual fetal eye movement can be observed by the use of real-time ultrasound. Almost at the same time, Birnholz² noted four types of fetal eye movements with their characteristic sequence of development. Subsequently, Nijhuis et al.³ and Precht and Nijhuis⁴ studied the biologic implications of fetal eye movement in relation to the fetal behavioral state, referring to the criteria of the neonate established by Precht.⁵ We investigated chronologic variations of fetal eye movement patterns with advance in gestational age. This approach allows for interpretation of mechanisms related to the process of development, on which the fetal eye movement depends.^{3,7}

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Material and methods

Observation of fetal eye movement by real-time ultrasound. As shown in Fig. 1, real-time ultrasound reveals the eyeball as a round-shaped area in the forehead of the fetus. Here, taking into account the anatomic architecture, structures such as the eyelid, cornea, lens, and corpus vitreum can be differentiated.

The lens in particular can be clearly identified in the form of a pair of small dotted echoes, probably originating from both the near and the far margins of the lens, so that it is considered most suitable as a landmark for observing positional change due to eye movement.

The observational standard plane was determined to be a nearly horizontal transection of the fetal head, including both orbits, in which the lens can most clearly be seen. One unit of eye movement was defined as that in which the lens proceeds from a stationary position to a moving one. The ultrasound equipment used was an ALOKA SSD-256 electronic scanner with a linear array transducer of 3.5 MHz.

Population of fetuses. The data presented here were derived from fetuses of mothers being cared for in the Department of Gynecology and Obstetrics, Faculty of Medicine, Kyushu University. All gave informed consent to participate in this study.

The gestational age was assessed according to the dates of the last menstrual period and also by means of serial ultrasound measurements of either the crown-rump length before 11 weeks or the biparietal diameter at 12 weeks or more. Two series of fetuses were investigated as follows.

Continuous observation of eye movement. A total of 28

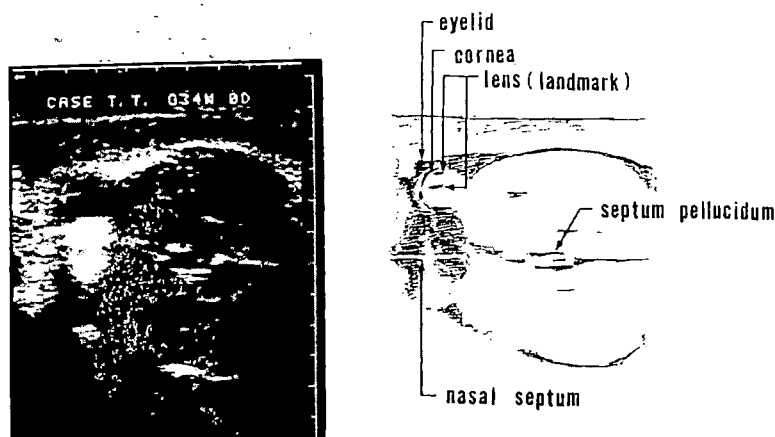


Fig. 1. Echographic image and illustration of the fetal eye in a transverse section of the head.

normal fetuses between 18 and 42 weeks of gestation were continuously observed for eye movements. Observations lasted from 45 to 110 minutes, under conditions where the mother was in the semi-Fowler position and a clear image of the fetal eyes could be readily obtained. During these periods, real-time ultrasound images were stored on videotapes. While these videotapes were being replayed, an attempt was made to calculate the frequency of eye movement at each 1-minute epoch.

Cross-sectional observation of eye movement. We obtained data on 217 cross sections of 209 fetuses from 18 to 41 weeks' gestation. The frequency of eye movement was regarded as that at any given 1-minute epoch during about a 5-minute observation period.

Results

Chronologic changes of time-related fetal eye movements. Fig. 2 shows findings taken from continuous observations arranged in ascending order of gestational age, where the horizontal and vertical axes indicate every 1 minute of observation and the frequency of eye movements at the corresponding time interval, respectively.

The length of discontinuity on the horizontal axis means the period during which the fetal eye itself could not be visualized by ultrasound.

At around 20 weeks of gestation, the onset of fetal eye movements is sporadic and the absence of movements is considerable. There is also a noticeable lack of movement with a frequency exceeding 5 per minute.

However, eye movements not only increase but begin to consolidate at about 24 weeks, after which this tendency becomes more distinct and consolidation grows into long-term clusters of eye movement, particularly at around 30 weeks onward.

There appears to be no relation in occurrence between the absence and the presence of movements at

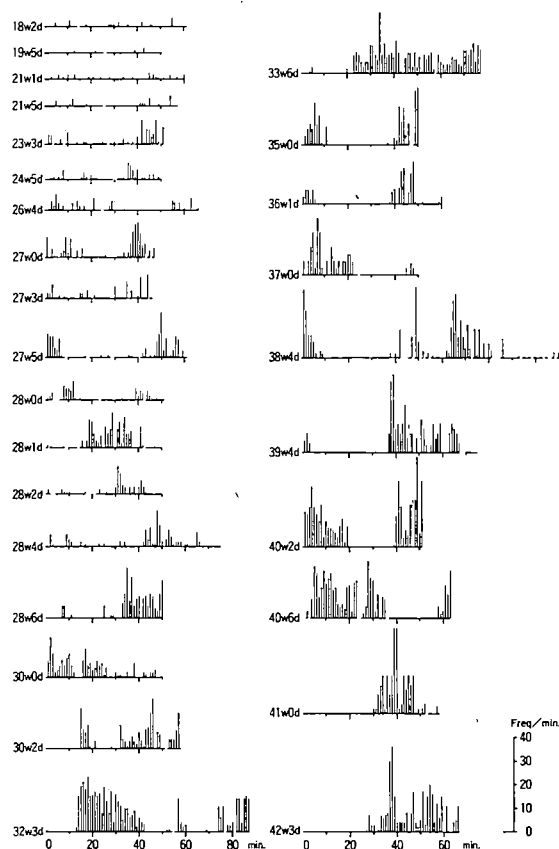


Fig. 2. The chronologic sequences of fetal eye movement, obtained from continuous observations from 45 to 110 minutes, where the horizontal and the vertical axes indicate the 1-minute epoch and the corresponding frequency of the eye movement, respectively. The elapsed area on the horizontal axis indicates the period when the fetal eye could not be observed.

<35 to 36 weeks of gestation. These two periods begin to alternate and result in a state of periodicity of fetal eye movements at around 36 weeks. At this time, both the sporadic onset and the low-frequency eye move-

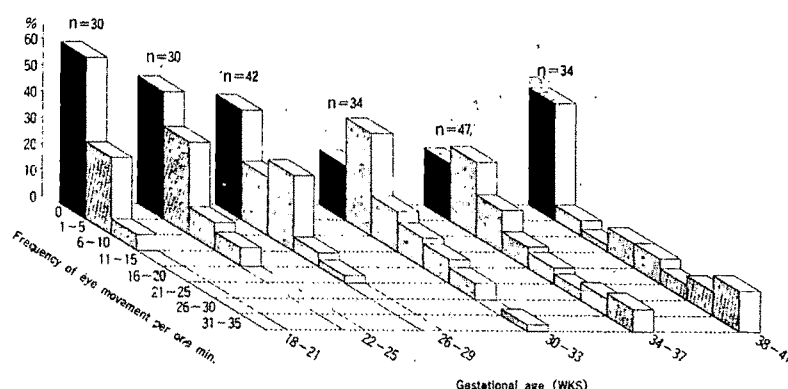


Fig. 3. Histograms obtained every 4 weeks from 18 to 41 weeks of gestation, together with the frequency of eye movement at a given minute during a 5-minute observation period and the corresponding cumulative incidence, expressed as a percent. These data are from a cross-sectional study.

ment, characteristic of cases at as early as about 20 weeks, disappear.

This finding is clearly evident together with a rapid increase in the frequency of movement from 38 weeks to term, where the absence and presence periods range from approximately 20 to 30 and from 30 to 40 minutes, respectively.

Chronologic changes in frequency of fetal eye movements. An increase in fetal eye movements with a slight variance in the frequency was noted at 18 weeks, followed by a definite tendency toward a wider variance, with the advance in gestation.

Fig. 3 shows the cross-sectional data of eye movement on a scale of gestational age, with the use of six histograms; including data for every 4 weeks of gestation, from 18 to 41 weeks. Initially, as for the frequency of "zero," indicating that no eye movements take place at a given 1-minute epoch, a particular finding is that the corresponding incidences decline in turn with percent values of 60, 48, 42, and 19, respectively, on the histograms obtained at 18 to 21, 22 to 25, 26 to 29, and 30 to 33 weeks.

Conversely, on the two histograms obtained at 34 to 37 and 38 to 41 weeks, the above incidences again increase with values of 22% and 44%, respectively.

With respect to epochs in which fetal eye movements are present, ranges of the frequency of movements increase progressively up to 30 to 33 weeks, after which they remain constant up to term.

These findings are summarized, on the basis that the frequencies of fetal eye movements can be classified into three groups of 1 to 10 (low), 11 to 20 (moderate), and 21 to 35 (high), as follows: Low-frequency eye movements can be seen at 18 to 21 weeks and much the same incidences are evident up to 34 to 37 weeks, although they do decrease sharply at ≥ 38 weeks.

Eye movements of moderate frequencies begin to increase at 22 to 25 weeks and continue to do so up to 30 to 33 weeks. Thereafter, there is little change in the values, irrespective of the advance in gestational age. Inversely, high-frequency eye movements commence at 30 to 33 weeks with progressive expansion of the incidence along with the advance in gestation and are a major component of eye movements at term.

These data are compatible with the frequencies of movements observed from the continuous observations shown in Fig. 2.

Comment

Following extensive studies on the behavioral states of the newborn infant,⁵ there has been a growing interest in the development of behavior in the human fetus. In this regard, the heart rate and various types of movement, including those felt by the mother, and respiratory and eye movements visualized by real-time ultrasound were used for analysis,⁸⁻¹² since electroencephalograms and electrooculograms have no in utero application.

To better comprehend the ontogenesis of fetal behavioral states, individual parameters must be descriptive so as to assess the biologic and underlying implications of the developmental process. From this point of view, we investigated the profile of in utero fetal eye movement with the use of real-time ultrasound. In agreement with reports of Bots et al.¹ and Birnholz,² we found that fetal eye movement could be observed from around 16 weeks through term, in all except one fetus in the prone position and during the period when there were active fetal movements, especially head-rolling movements. Nevertheless, approximately less than 3% of all the periods showed these related conditions. In contrast with Bots et al.,¹ who evaluated eye move-

ment by observing small movements in the retroocular area, we used a pair of echoes of the lens as a landmark for analysis, because these echoes were expressed on the echographic image in the form of small dots. Here a more exact positional deviation was detectable.

Fitting these data to those of the Birnholz classification² demonstrates that the frequencies of fetal eye movements, low, moderate, and high, are comparable to types I, II-III, and IV, respectively. All the data are in good accord with regard to onset of gestational age, at which all types of movement begin to develop, with the related sequential features.

However, only with regard to the timing of withdrawal of the low-frequency eye movement, our data indicate such event at 34 to 37 weeks, while Birnholz² reported evidence obtained at 25 weeks. Bridgeman¹³ stated that type I movements in the premature infant were replaced by normal-conjugate saccades alternating with fixation by 33 weeks of gestation.

Thus, when one considers that low-frequency eye movement is characteristic of a sporadic and random onset, as shown in Fig. 2, decrease in low-frequency eye movement would indicate an association by 34 to 37 weeks, even in the human fetus, with maturation of the tonic system, by which the eye is held in an eccentric position.¹³

As moderate-frequency eye movements seem related to poor consolidation of eye movement, it is suggested that the transitional state proceeds to rapid eye movement, as shown in Fig. 2. As rapid eye movement in the newborn infant can be defined as a frequency of eye movement of >20 per minute,⁵ the high-frequency eye movement we observed in fetuses is probably rapid eye movement.

Accordingly, rapid eye movement seemed to be first manifested at 30 to 33 weeks' gestation (Fig. 3). This is in good agreement with the proposal that changes in heart rate patterns related to the rest-activity cycle of the fetus seen from 34 weeks may reflect the development of rapid eye movement and non-rapid eye movement sleep.⁹

The frequencies of no eye movement run a most peculiar course, with respect to chronologic changes. The histogram taken at 38 to 41 weeks of gestation has a bimodal property, indicative of no eye movement and a high-frequency eye movement. In addition, these two types of eye movements are found to consolidate at around 30 weeks and clearly occur in an alternative fashion at about 35 to 36 weeks, as shown in Fig. 2. Consequently, since this lack of eye movements and presence of high-frequency eye movements probably correspond to so-called non-rapid eye movement and rapid eye movement sleep, respectively, these two sleep states may begin to develop during 34 to 37 weeks of

gestation and at the latest by 38 weeks, the mechanism related to regulation of sleep apparently reaching maturation.

The ranges of periods of absence and presence of fetal eye movement are approximately from 20 to 30 and from 30 to 40 minutes in the near-term fetus. This is compatible with the mean durations of non-rapid eye movement and rapid eye movement states, respectively, noted in an analysis of heart rates in the term fetus.^{11, 12}

Thus, a high incidence of no eye movement near term has essentially a different biologic meaning, compared with that in early pregnancy, especially in cases of less than 29 weeks' gestation, where no eye movement would imply an immature oculomotor system and lead to a long-term period of lack of movement.

The chronologic profile of our data with respect to eye movements between 32 and 41 weeks of gestation supports the data of Nijhuis et al.³ and Prechtl and Nijhuis⁴ that rapid and slow eye movements are organized in episodes of activity and become linked with other variables (for instance, breathing movement or fetal heart rate pattern) into coherent behavioral states at about 36 to 38 weeks of gestation.

The approaches described in this report led to a quantitative analysis of fetal eye movement in utero. This will pave the way for studies related to parameters such as fetal heart rate.

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The relative fatty acid composition of serum lecithin and cholesterol ester: Influence of an estrogen-progestogen regimen in climacteric women

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The relative fatty acid compositions of serum lecithin and cholesterol ester were studied during continuous treatment with an estrogen/progestogen combination. All 26 women who participated suffered from climacteric complaints and were given one tablet daily containing 2 mg of 17 β -estradiol, 1 mg of estriol, and 1 mg of norethindrone acetate. Blood samples were taken before treatment and after 3 and 12 months of treatment. In serum lecithin, a decrease ($p < 0.01$) in stearic acid concomitant with an increase of linoleic ($p < 0.05$) and arachidonic ($p < 0.01$) acids was recorded. The most evident changes noted in cholesterol ester were decreased levels of stearic ($p < 0.01$), oleic ($p < 0.05$), and arachidonic ($p < 0.001$) acids. Polyunsaturated acids of the n-6 series are precursors for prostaglandins and leukotrienes. In the present study an increase of dihomo- γ -linolenic, linoleic, and arachidonic acids in serum lecithin was found after treatment, and theoretically this finding may have an impact on the prostacyclin-thromboxane balance. (*AM J OBSTET GYNECOL* 1986;155:174-7.)

Key words: Fatty acids, estrogen, progestogen, menopause

Estrogens and progestogens are well known for their rapidly ameliorating effect on climacteric complaints, such as hot flushes and sweats experienced by women around the menopause. Different combinations of estrogens and progestogens have been advocated and the disadvantages of such regimens are inevitably bleeding disorders. Continuous estrogen-progestogen therapy does not lead to cyclic transformation of the endometrium, and therefore continuous therapy is of interest and recently has been shown to reduce bleeding episodes in postmenopausal women.¹

It is well established that sex steroids directly or indirectly influence lipid metabolism. Exogenous sex steroids have been found to redistribute the relative amounts of individual serum phospholipids.² The fatty

acid compositions of hepatic and serum phospholipids and serum cholesterol ester are also under dietary influence.³ Sex steroids are known to influence the excretory capacity of the liver.⁴ Redistribution of the fatty acid composition of lecithin and cholesterol ester in serum is a sensitive indicator of alterations in lipid metabolism induced by sex steroids.⁵

A number of studies during recent years indicate that changes in serum fatty acids might be of relevance for prostaglandin synthesis. It has been proposed that women with premenstrual syndrome may have abnormal essential fatty acid levels in the plasma.⁶ Women with a diet rich in eicosapentaenoic acid had prolonged bleeding times concomitant with a low incidence of thromboembolic diseases. When eicosapentaenoic acid was added to a normal western diet, its content in serum phospholipids increased and furthermore there was a decrease in platelet aggregation and a prolonged bleeding time.⁷ The prostacyclin-thromboxane balance is of special importance in regard to the risk of developing deep venous thrombosis after intake of sex steroids. Consequently, it may be of interest to study changes in

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Table I. Serum concentrations (mean $\pm$ SEM) of lecithin and cholesterol ester in climacteric women ($n = 26$) before treatment and after 3 and 12 months of estrogen-progestogen treatment

	Before	At 3 mo	At 12 mo
Lecithin (mmol/L)	2.51 ± 0.10	$2.21^* \pm 0.10$	2.38 ± 0.11
Cholesterol ester (mmol/L)	4.50 ± 0.18	$3.97^* \pm 0.16$	4.09 ± 0.16

* $p < 0.05$ compared with pretreatment value.

precursor fatty acids in women after administration of sex steroids. Moreover continuous estrogen-progestogen therapy for women in the climacteric period is new and its metabolic effects should be evaluated.

Material and methods

Patients and treatment. Twenty-six healthy women with a mean age of 50.3 years (range 43 to 59) and presenting with sweats and hot flushes, were treated with an estrogen-progestogen combination for 12 months. Each patient received one tablet daily consisting of 2 mg of 17β -estradiol, 1 mg of estriol, and 1 mg of norethindrone acetate. No other drugs were given for at least 4 weeks before the study. All of the women participated in the study after informed consent and the Ethics Committee of the University of Göteborg approved the study design. Body weights were measured and blood samples were obtained before treatment and after 3 and 12 months of treatment. Blood samples were collected between 8 and 10 AM after an overnight fast at different periods of the year and no seasonal variations should therefore affect the results. All women were asked about dietary habits, which appeared to be reasonably normal, but no detailed history was taken. For further clinical details see reference 1.

Serum lecithin and cholesterol ester analyses. Lipids were separated by thin-layer chromatography on silica gel for the isolation and determination of lecithin, while fatty acid methyl esters were prepared from the lecithin according to Olegård and Svennerholm.⁸ Cholesterol esters and cholesterol fatty acid methyl esters were prepared as described by Crona et al.⁹ The fatty acid methyl esters were analyzed by gas-liquid chromatography on a Perkin Elmer Model 30 gas chromatograph and the relative composition (moles per 100 ml) of fatty acids was determined. Only fatty acids of more than 1% were considered and only the important ones were tabulated.

Statistical analysis. Conventional methods were used for calculation of the mean, standard deviation, and standard errors of the mean. Student's t test was used

Table II. Relative composition of some major fatty acids of serum lecithin in climacteric women ($n = 26$) before treatment and after 3 and 12 months of estrogen-progestogen treatment. Values are moles per 100 ml of methyl esters (mean $\pm$ SEM)

Fatty acid	Before	At 3 mo	At 12 mo
16:0	32.26 ± 0.38	32.89 ± 0.37	32.94 ± 0.49
18:0	15.47 ± 0.22	$12.35 \pm 0.24^*$	$12.51 \pm 0.21^*$
18:1	12.59 ± 0.25	13.11 ± 0.27	$13.31 \pm 0.25^\dagger$
18:2 n-6	23.99 ± 0.51	$25.01 \pm 0.65^\dagger$	$25.03 \pm 0.60^\dagger$
20:3 n-6	2.51 ± 0.12	2.64 ± 0.13	$3.08 \pm 0.16^\dagger$
20:4 n-6	6.42 ± 0.26	$7.36 \pm 0.29^*$	$7.04 \pm 0.29^*$

* $p < 0.01$ compared with pretreatment value.

$^\dagger p < 0.05$ compared with pretreatment value.

16:0 = Palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 20:3 = dihomo- γ -linolenic acid; 20:4 = arachidonic acid.

Table III. Relative composition (moles per 100 ml) of some major fatty acids of serum cholesterol ester (mean $\pm$ SEM) in climacteric women before treatment and after 3 and 12 months of estrogen-progestogen treatment

Fatty acid	Before	At 3 mo	At 12 mo
16:0	11.73 ± 0.29	$12.64 \pm 0.21^*$	10.72 ± 0.47
18:0	1.29 ± 0.05	$0.90 \pm 0.04^\dagger$	1.29 ± 0.06
18:1	22.05 ± 0.42	$20.79 \pm 0.56^*$	23.41 ± 0.54
18:2	53.97 ± 0.76	54.88 ± 1.08	54.53 ± 0.78
20:4	5.22 ± 0.21	$4.69 \pm 0.22^\dagger$	$4.68 \pm 0.25^\dagger$

For explanation of fatty acids see footnote to Table II.

* $p < 0.05$ compared with pretreatment value.

$^\dagger p < 0.01$ compared with pretreatment value.

for calculation of statistical significance. A p value < 0.05 was considered statistically significant.

Results

Almost all the women were completely free from vasomotor complaints after 3 months of treatment. Body weight was 63.1 ± 6.2 kg (mean $\pm$ SD) before treatment. After 3 and 12 months of treatment body weights were 64.1 ± 6.6 and 63.8 ± 6.7 kg.

The absolute values of lecithin and cholesterol ester before treatment and after 3 and 12 months of treatment are shown in Table I. The relative fatty acid compositions of serum lecithin and serum cholesterol ester before treatment and during treatment appear in Tables II and III.

In serum lecithin a decrease ($p < 0.01$) in stearic acid was noted after 3 and 12 months of treatment. Increases of linoleic ($p < 0.05$) and arachidonic acids ($p < 0.01$) were recorded after 3 and 12 months and oleic acid also increased ($p < 0.05$) after 12 months.

In cholesterol ester, an increase ($p < 0.05$) was seen

in palmitic acid after 3 months concomitant with a decrease in stearic ($p < 0.01$), oleic ($p < 0.05$), and arachidonic acids ($p < 0.01$). When 3 and 12 months of treatment were compared, a decrease ($p < 0.01$) was noted for palmitic acid while increments were found in stearic ($p < 0.01$) and oleic acids ($p < 0.01$). The decrease in arachidonic acid after 3 months persisted after 12 months of treatment.

In serum lecithin, an increase to 3.08 ± 0.16 mol % ($p < 0.05$) was found compared to the pretreatment value in dihomo- γ -linolenic acid (20:3) after twelve months of treatment.

Comment

Lecithin is formed in the liver through two major pathways and remodelled through the Lands-van Deenen cycle.¹⁰ Predominantly saturated fatty acids are situated in the 1-position whereas unsaturated fatty acids dominate the 2-position. Changes in the relative fatty acid composition of serum lecithin seem to be a sensitive indicator of any change in liver metabolism. In serum lecithin linoleic acid is the primary precursor of most other important polyunsaturated fatty acids. Arachidonic acid can be obtained from the diet directly or by desaturation of linoleic acid to γ -linolenic acid, followed by chain elongation to dihomo- γ -linolenic acid and then further desaturation.¹¹ A change in the relative amount of these fatty acids could therefore theoretically either be due to a disturbance in these metabolic events or be of dietary origin.

A significant increase in the palmitic-stearic acid ratio in serum lecithin was found in the present investigation and similar results have been reported previously whenever carbon 17-acylated compounds are given, regardless of their hormonal characteristics. This finding may be associated with an inhibited hepatic excretory function.^{4,5} An increase in the major essential fatty acid linoleic acid has been reported after addition of norethindrone acetate to estrogens.⁵ Furthermore a decrease in lecithin after 3 months was seen in the present study, also confirming previous results obtained after treatment with 19-nortestosterone derivatives.⁵ However, in studies using higher doses of synthetic progestins with some androgenic activity, a decrease in arachidonic acid concomitant with a linoleic acid increase has been reported.¹² Therefore increments of linoleic and arachidonic acids in serum lecithin may be caused partly by a dietary influence since progestins of the 19-nortestosterone type may have anabolic effects. Slight increments in body weights, although not significant, were found in the present group of women during treatment. It was noted that modulation of diet in terms of increased carbohydrate and protein intake may stimulate the enzyme Δ -6-desaturase in the synthesis of dihomo- γ -linolenic acid.¹³ The possible dietary effect af-

ter 3 months of treatment was seen also in cholesterol ester as judged from an increase in palmitic acid and decreases in oleic and arachidonic acids. An adaptation to pretreatment values, except for arachidonic acid, was found after 12 months of treatment.

The incidence of venous thrombosis in women is increased with the use of oral contraceptives and a similar trend may be evident in postmenopausal women treated with estrogenic hormones.¹⁴ Prostacyclin (prostaglandin I_2) and thromboxane A_2 , like other prostaglandins, are formed from the precursor fatty acids dihomo- γ -linolenic acid, arachidonic acid, and eicosapentaenoic acid. It has been shown that the microsomal fractions of the vessel wall are able to generate a potent platelet antiaggregation compound, prostaglandin I_2 , from prostaglandin endoperoxides and arachidonic acid.¹⁵ Thromboxane A_2 is a product of the platelets and characterized by vasoconstrictory and proaggregatory properties.¹⁶ The present combination seems to make precursors for prostaglandins more readily available in terms of increased levels of polyunsaturated fatty acids of the n-6 series. The further fate of these fatty acids, however, cannot be inferred from the present study. The unsaturated fatty acid content of cholesterol ester did not mirror the composition of lecithin but, since both lecithin and cholesterol ester were reduced in a similar ratio after 3 and 12 months, it seems reasonable to conclude that the lecithin-cholesterol acyltransferase reaction was rather unaffected by treatment.

Several studies have been performed to evaluate the metabolic effects of prostaglandin I_2 and A_2 . In rodents, most studies indicate that vessel walls increase the production of prostacyclin when they are under the influence of exogenous estrogens. Norethindrone acetate did not influence the ability of the female rat thoracic aorta or inferior vena cava to produce prostacyclin when administered alone,¹⁷ and in general the information regarding progestogens is very scarce. Ylikorkala et al.¹⁸ found a decreased capacity of platelets to synthesize A_2 during clotting in women using progestogen-only oral contraceptives, which theoretically could result in a reduced risk of thrombosis formation. This progestogen-induced suppression in proaggregatory A_2 formation was compatible with the finding that norethindrone could prevent the increase of platelet aggregation induced by ethinyl estradiol in rabbits in vivo.¹⁹ It seems reasonable that exogenous estrogens and progestogens may have opposing effects on the aggregation of platelets and on the prostacyclin-thromboxane balance. These effects can theoretically be modified by changes in the availability of different precursor fatty acids that modulate phospholipase activities and other steps essential in the biosynthesis of these prostanoids. However, further investigations are war-

ranted before the patterns of sex steroid influence on these systems can be fully understood.

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Recurrent carcinoma in situ of the vulva in a skin graft

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Skinning vulvectomy has been a recommended treatment for carcinoma in situ of the vulva. We report a case of recurrent carcinoma in situ occurring in the skin graft of a patient with previous skinning vulvectomy. (*AM J OBSTET GYNECOL* 1986;155:177-9.)

Key words: Vulva, carcinoma in situ, skinning vulvectomy

During the past two decades the incidence of vulvar carcinoma in situ has increased. Currently it is diag-

nosed more frequently in younger women, is commonly multifocal, and rarely progresses to invasive cancer. The treatment of choice had been considered to be total vulvectomy with primary closure until 1968, when Rutledge and Sinclair¹ recommended skinning vulvectomy to replace the skin at risk with ectopic dermis. More recently wide local excision or laser removal of localized lesions has been recommended as therapy. No cases of recurrent vulvar carcinoma in situ on the

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Fig. 1. Several slightly raised, pigmented areas present on the surface of the grafted skin.

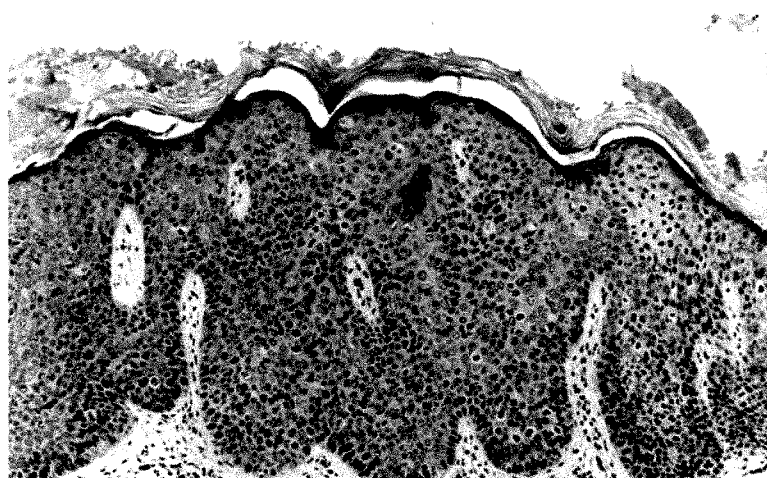


Fig. 2. Biopsy specimen taken from pigmented area seen in Fig. 1, compatible with squamous cell carcinoma in situ. (Hematoxylin and eosin stain. Original magnification $\times 100$.)

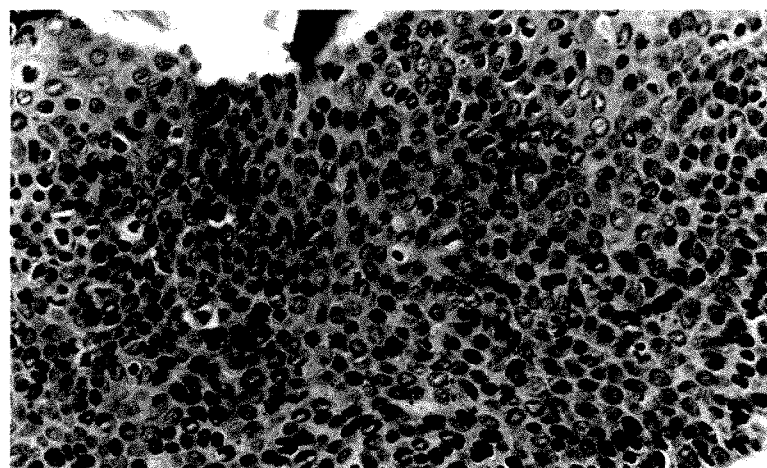


Fig. 3. Higher-power view of Fig. 2, demonstrating enlargement, variability, and hyperchromatism. (Hematoxylin and eosin stain. Original magnification $\times 250$.)

grafted skin replacing the diseased tissue have been reported. However, Lathrop et al.² recently reported two cases of recurrent vaginal carcinoma in situ in a split-thickness skin graft. We are reporting a case of recurrent vulvar carcinoma in situ occurring in the skin graft of a patient 7 years after skinning vulvectomy with graft. This report represents the third case of recurrent carcinoma in situ in ectopic skin.

Case presentation

M. C., a 30-year-old, nulliparous, Hispanic patient, status post splenectomy for idiopathic thrombocytopenic purpura, had a long-standing history of cervical, anal, and vulvar carcinoma in situ. She originally presented in 1977 with an abnormal Papanicolaou smear; an adequate colposcopic examination and cervical biopsy specimens demonstrated squamous cell carcinoma in situ. She underwent cryocautery, and follow-up Papanicolaou smear at 3 months revealed moderate dysplasia. The patient was then treated with a cervical conization that revealed squamous cell carcinoma in situ. During this time she developed condyloma acuminatum refractory to podophyllin. She subsequently underwent excisional biopsy and electrocautery treatment. The biopsy specimen revealed squamous cell carcinoma in situ of the vulva, and in February, 1978, she was treated with skinning vulvectomy with grafts taken from the buttocks. The pathology report revealed multifocal squamous cell carcinoma in situ, with anal margins free and periurethral margins having moderate dysplasia. Since that time the patient has had recurrent multifocal vulvar carcinoma in situ especially in the perianal area. These new lesions have been treated with excisional biopsy or laser therapy. In 1985, the patient

presented with pigmented lesions located centrally in the graft site (Fig. 1). Biopsy of these lesions revealed squamous cell carcinoma in situ (Figs. 2 and 3).

Comment

Since its conception, skinning vulvectomy, as a treatment for multifocal vulvar carcinoma in situ, has met with some resistance. The susceptibility of the donated skin to similar neoplastic processes has been questioned. Lathrop et al.² reported two cases of recurrent vaginal carcinoma in situ in neovaginas less than 18 months after graft placement. These authors believed that an environmental oncogenic stimulus reacted with neovaginal tissue to produce the dysplastic changes.

To our knowledge this is the first case reported of recurrent vulvar carcinoma in situ in the donated skin. This and other case reports illustrate the importance of close observation and biopsy of all suspicious lesions in these patients. Furthermore, if similar cases are diagnosed, the rationale for skinning vulvectomy should be reevaluated. This case further supports the belief of Lathrop et al.² that the etiologic factors responsible for the development of intraepithelial neoplasia may persist after treatment and thus be responsible for recurrence of disease, even in new grafted skin.

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Personal protection against sexually transmitted diseases

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Primary prevention of sexually transmitted diseases has received little emphasis in control programs. To evaluate the effectiveness of prevention strategies, we reviewed the world's literature on this topic. Use of condoms and spermicides greatly reduces the risk of gonorrhea, and barrier methods are practical and acceptable. Certain systemic antibiotics are effective, but for practical reasons, their use cannot be recommended. Washing or urinating after sexual exposure does not appear to protect against infection. Although the effectiveness of modifying sexual behavior to reduce the risk of sexually transmitted diseases has not been evaluated, many people have changed or are willing to change their behavior. Even if preventive measures are used consistently by only a minority of persons at risk, rates of sexually transmitted diseases would decline rapidly. In this era of sexually transmitted diseases that are not readily cured by antibiotics, use of effective preventive measures should be strongly encouraged. (AM J OBSTET GYNECOL 1986;155:180-8.)

Key words: STD, prevention, barriers

Reliance on containment rather than on primary prevention of sexually transmitted diseases (STD) has hampered control programs in the United States. Early detection of disease, appropriate treatment, and contact tracing are cornerstones of contemporary control strategies, yet these emphasize secondary prevention, that is, limitation of morbidity once disease is present. Except in military populations during wartime,¹ strategies for personal protection against STD have been regarded unfavorably because of dubious effectiveness and concerns about compliance.

As the range of STD has expanded from bacterial to viral pathogens [hepatitis B, herpes simplex, human papillomavirus, and human T-lymphotropic virus type III (HTLV-III)], the assurance of prompt cure has vanished. Concern about "safe sex" is growing, and some segments of the population are adopting personal protection strategies to reduce their risk of STD.^{2,3} Moreover, both laboratory and clinical data in recent years have provided much-needed documentation of the effectiveness of several personal protection strategies. In this article, we will provide an overview of these strategies, summarize selected efficacy studies, and make general recommendations for personal protection against STD.

Methods

A large number of studies since World War I have described strategies for reducing one's risk of acquiring STD; most of these studies have been reviewed elsewhere.⁴ We review here those studies retrieved through computerized searches that provided sufficient detail to assess efficacy. To provide an overview of the findings, we have derived or abstracted from each study a measure of the protective effect of the strategy under question and calculated a 95% confidence interval for this measure. All the measures of effect are equal to 1.0 if the strategy has no effect, <1.0 if there is a protective effect, and >1.0 if the strategy increases the risk of disease.

In most studies, the measure used can be regarded as a relative risk. For example, a measure of 0.5 suggests that the risk of disease among those using the strategy is half the risk in those not doing so. In case-control studies, the measure of effect is an estimate of the odds ratio—the odds of getting the disease among those using the strategy divided by the odds of getting the disease among those not using the strategy. For some of the follow-up studies, the measure of effect is the ratio of the disease rates (incidence density) in the two groups. Confidence intervals were derived by means of Miettinen's test-based approach.⁵ The 95% confidence interval indicates the precision of the measure of effect; the narrower the interval, the greater the precision. If the 95% confidence interval does not include 1.0, the effect is statistically significant at the 0.05 level.

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Table I. Efficacy of condom use in preventing sexually transmitted diseases

<i>Study</i>	<i>Study design</i>	<i>Study population</i>	<i>Outcome</i>	<i>Measure of effect</i>	<i>95% Confidence interval</i>
Pemberton et al., 1972 ⁷	Cross-sectional	Males, STD clinic, Belfast	Urethral gonorrhea	0.51	0.33-0.80
McCormack et al., 1973 ⁹	Cross-sectional	Male college volunteers, United States	Nongonococcal urethritis	1.20	0.90-1.59
Darrow, 1973 ¹⁴	Cohort	STD clinic attendees, California	Urethral T-mycoplasma colonization	0.33	0.16-0.68
Hart, 1974 ¹⁰	Cohort	Australian soldiers, Vietnam	Urethral gonorrhea	0.34	0.10-1.13
			Self-reported venereal diseases	0.00*	
Barlow, 1977 ⁸	Cross-sectional	Males, STD clinic, London	Urethral gonorrhea	0.25	0.11-0.59
Hooper et al., 1978 ¹⁵	Cohort	Naval crewmen, Far East	Nongonococcal urethritis	0.85	0.69-1.07
			Urethral gonorrhea	0.00†	
Kelaghan et al., 1982 ¹¹	Case-control	Hospitalized women, United States	Pelvic inflammatory disease	0.6	0.4-0.9
Austin et al., 1984 ¹²	Case-control	Females, STD clinic, Alabama	Cervical gonorrhea	0.87	0.64-1.19
Syrjanen et al., 1984 ¹³	Case-control	Females, obstetrics and gynecology clinic, Finland	Cervical human papilloma virus infection	1.35	0.72-2.78

*p < 0.0001, Fisher's exact test (none of 55 condom users were infected; 26 of 96 using no prophylaxis were infected).

†p < 0.1, Fisher's exact test (none of 29 condom users were infected; 51 of 498 nonusers were infected).

Results

Restriction of sexual activities. Avoiding sexual partners who are likely to be infected and limiting the number of partners should be effective ways to prevent acquisition of sexually transmitted pathogens. In one study⁶ of adolescent men with recurrent gonorrhea, those who remained free of infection during a 9-month period had reduced their number of partners, whereas those who became reinfected had not. However, the effect of behavioral changes on STD risk reduction has not been adequately studied.

Condoms. If used consistently and correctly, condoms are considered to be highly effective in preventing acquisition and transmission of most STD. Efficacy has been inferred from several studies (Table I) that found lower infection rates among correct users as compared with nonusers and incorrect users. Measures of the protective effect of condom use ranged from 0 (total protection) to 1.2.

Among men attending an STD clinic in Belfast for the first time, a cross-sectional study⁷ found that condom users were much less likely than nonusers to be infected with gonorrhea. Another study⁸ of men attending an STD clinic in London found that gonorrhea was less common among correct users of condoms as compared with nonusers. Neither of these studies showed a protective effect of condom use on nongonococcal urethritis; however, such an effect might have been obscured in these STD clinic populations if condoms are protective against gonorrhea.

Asymptomatic male college students who "always" or "almost always" used condoms were significantly less likely to be colonized with *T-mycoplasmas* than those who "seldom" or "never" used condoms.⁹ This effect per-

sisted even when the number of sexual partners was taken into account. Among Australian soldiers returning from Vietnam, those who reported using condoms were significantly less likely to report having had "venereal diseases" than nonusers.¹⁰ However, condom users were more likely to be married than were nonusers, and confirmation or specification of self-reported diseases was not attempted.

Condoms may also protect women from STD. A large United States multicenter case-control study¹¹ suggested that women relying on condoms for contraception had a significantly lower risk of hospitalization for pelvic inflammatory disease than did women using no contraception. Although the difference was not significant, a case-control study¹² showed that female STD clinic attendees whose partners had used condoms in the previous 3 months were less likely to be infected with gonorrhea, compared with those whose partners had not used condoms. No significant differences in condom use were observed between Finnish women with cervical human papillomavirus infections and randomly selected women who had normal cervical Papanicolaou smears.¹³ However, the sexual behavior of women in the two groups differed significantly; the numbers of sexual partners, sexual exposures, and previous STD were greater for human papillomavirus-infected women compared with control women.

Two prospective cohort studies investigating condom efficacy have been published. In one,¹⁴ free condoms were distributed to male STD clinic attendees desiring condoms; then a random sample of condom recipients was examined 3 months later. Although the men who reported always using condoms were less likely to be infected with gonorrhea than those who had never used

Table II. Efficacy of contraceptive barrier use in preventing sexually transmitted diseases in women

Study	Study design	Study site	Barrier	Outcome	Measure of effect	95% Confidence interval
Berger et al., 1975 ²²	Cross-sectional	Family Planning Clinic, Louisiana	Condom/diaphragm/foam	Cervical gonorrhea	0.53	0.18-1.59
Kelaghan et al., 1982 ¹¹	Case-control	16 hospitals, United States	Diaphragm	Pelvic inflammatory disease	0.4	0.2-0.7
Austin et al., 1984 ¹²	Case-control	STD clinic, Alabama	Diaphragm	Cervical gonorrhea	0.45	0.12-1.67
Quinn and O'Reilly, 1985 ²¹	Cross-sectional	STD clinic, Tennessee	Condom/diaphragm	Cervical gonorrhea	0.11	0.08-0.17

condoms, the difference was not statistically significant. In the other study,¹³ involving naval crewmen who had engaged in sexual activity while in port, none of the 29 men who "always" or "sometimes" used condoms acquired gonorrhea, but 51 of 498 (10%) men who did not use condoms acquired gonorrhea. This protective effect of condoms does not achieve statistical significance, due in part to the small numbers of condom users. Ascertainment bias may have occurred, in that only symptomatic men had cultures for gonorrhea on their return to the ship.

Although generally encouraging, these studies, like most observational studies, must be interpreted with caution. Men who use condoms consistently are likely to differ from those who do not. Most studies have not compared users and nonusers in regard to demographic variables, specific sexual practices, frequency of sexual activity, frequency of exposures to infected partners, use of other prophylactic measures, and technical adequacy of condom use.

In vitro condom studies. An intact condom is generally trusted as a mechanical barrier to the passage of spermatozoa and bacteria. However, because of the much smaller size of viruses, questions persist about the efficacy of the condom as a barrier to these particles. Although transmission and scanning electron microscopy on a stretched and an unstretched latex condom¹⁶ showed numerous raised superficial irregularities in the latex, no pores or other defects compromising the integrity of the condom were observed.

Several in vitro studies have explored the possibility that a virus could pass through an intact condom into surrounding tissue culture media. These experimental transmission models, some of which simulated coitus, showed that condoms are effective barriers to herpes simplex virus type 2,¹⁷⁻¹⁹ *Chlamydia trachomatis*,¹⁹ cytomegalovirus,^{20a} and HTLV-III.^{20b}

Diaphragms and other barrier contraceptives. Several studies have found that barrier methods of contraception, including condoms, diaphragms, and spermicides, protect the user against STD (Table II). Compared with women not using contraception, women in

an STD clinic²¹ who used barrier contraceptives (condom or diaphragm) and women in a family planning clinic²² who used barriers (condom, diaphragm, or foam) were 90% and 50%, respectively, less likely to be infected with gonorrhea. Although neither of these reductions was statistically significant, neither study controlled for sexual activity. In one of two studies that did control for sexual activity, diaphragm users had a significantly lower risk of hospitalization for pelvic inflammatory disease compared with women using no contraception.¹¹ In the other, women in an STD clinic¹² who used diaphragms were less likely to have gonorrhea than were nonusers of barrier contraceptives (condom, diaphragm, or spermicide); however, this difference was not statistically significant. Although these data suggest a protective effect of diaphragm use on pelvic inflammatory disease and gonorrhea, the findings must be interpreted with caution, since diaphragms are almost always used with spermicides and, as will be discussed, spermicides alone may protect the user from STD.

Cervical cancer behaves epidemiologically like an STD.^{23, 24} Several studies have shown that women using condoms or diaphragms are less likely to develop cervical cancer or its precursors than are other women. In a British case-control study,²⁵ the relative risk of severe cervical dysplasia among condom and diaphragm users dropped from 1.0 to 0.2 during a 10-year period of barrier use. Another British case-control study²⁶ showed that the relative risk of cervical neoplasia for diaphragm users compared with users of other contraceptives was 0.2.

Spermicides. Vaginal spermicides used alone appear to lower the risk of acquiring gonorrhea (Table III). Measures of effect range from 0.1 to 0.9. In the largest randomized clinical trial,²⁷ women recently treated for gonorrhea who were assigned to receive nonoxynol 9 foam for 6 months had a significantly reduced rate of reinfection which was 10 times lower than that of the control group. In a small double-blind randomized clinical trial,²⁸ two of 24 (8%) women who had used phenylmercuric acetate suppositories and four of 24 (17%)

Table III. Efficacy of spermicides in preventing sexually transmitted diseases in women

Study	Study design	Study site	Spermicide	Outcome measure	Measure of effect	95% Confidence interval
Cutler et al., 1977 ²⁷	Randomized clinical trial	STD clinic, Pennsylvania	Nonoxynol 9	Cervical gonorrhea	0.11	0.02-0.55
Rendon et al., 1980 ²⁸	Randomized clinical trial	Outpatient clinic, Mexico	Phenylmercuric acetate	Cervical gonorrhea	0.30	0.08-1.14
Cole et al., 1980 ²⁹	Community trial	STD clinic, Florida	Nonoxynol 9	Cervical gonorrhea	0.60	0.21-1.73
			Phenylmercuric acetate	Cervical gonorrhea	0.51	0.32-0.80
Jick et al., 1982 ³⁰	Case-control	Health maintenance organization, Seattle	Octoxynol or nonoxynol 9	Cervical gonorrhea	0.13	0.04-0.40
Kelaghan et al., 1982 ¹¹	Case-control	15 hospitals, United States	Not specified	Pelvic inflammatory disease	0.7	0.4-1.4
Austin et al., 1984 ¹²	Case-control	STD clinic, Alabama	Not specified	Cervical gonorrhea	0.90	.47-1.73
Quinn and O'Reilly, 1985 ²¹	Cross-sectional	STD clinic, Tennessee	Not specified	Cervical gonorrhea	0.39	0.31-0.50

Table IV. Efficacy of local measures in preventing sexually transmitted diseases

Study	Study design	Population	Measure	Outcome	Measure of effect	95% Confidence interval
Edwards, 1973 ³⁵	Nonrandomized clinical trial	Prostitutes, Nevada	Orthoiodobenzoic acid	Cervical gonorrhea	0.08	0.02-0.37
Hart, 1974 ¹⁰	Cohort	Australian soldiers, Vietnam	Washing genitals	Urethral gonorrhea	1.52	0.87-2.7
Limsuwan et al., 1978 ³⁶	Nonrandomized clinical trial	Prostitutes, Thailand	Vaginal povidone-iodine	Cervical gonorrhea	0.25	0.15-0.42
Hooper et al., 1978 ¹⁵	Cohort	Naval crewmen, Far East	Washing genitals	Urethral gonorrhea	0.52	0.18-1.44
			Urination	Urethral gonorrhea	0.74	0.32-1.67

women who had used nonoxynol 9 suppositories became infected with gonorrhea, whereas eight of 24 (28%) women who had been given a placebo became infected during a 6-month follow-up. Although these results are suggestive of a protective effect of spermicides, neither of these reductions in gonorrhea was statistically significant.

In a community trial,²⁹ women recently treated for gonorrhea were given phenylmercuric acetate suppositories and observed for reinfection at monthly intervals for 3 months. The risk of reinfection in these women was 50% lower than that in a similar group of women who were not given suppositories and had been seen at the same clinic 3 months earlier. In a cross-sectional study, the risk of gonorrhea among women in an STD clinic who used spermicides alone was 60% lower than that for women not using contraceptives²¹; this difference was statistically significant.

Three recent case-control studies have demonstrated a protective effect of spermicides on STD. In one study,³⁰ women who used spermicides had one tenth the risk of having gonorrhea compared with women using oral or surgical contraception, a highly significant difference. Another large study¹¹ showed that those

women using spermicides alone had a 30% lower risk of hospitalization for first-episode pelvic inflammatory disease, compared with that of women not using contraceptives. This difference was not statistically significant. In another large study,¹² women who used spermicides alone had only a 10% reduction in the risk of gonorrhea compared with that of women not using condoms, diaphragms, or spermicides. However, when spermicides were used with either condoms or diaphragms, the risk of gonorrhea was reduced by 60%. Unlike the other case-control studies, this one controlled for the potentially confounding effects of differences in sexual activity.

Spermicides have been shown *in vitro* to inhibit the growth of both *Neisseria gonorrhoeae*^{31, 32} and *Trichomonas vaginalis*,³² to inactivate herpes simplex virus type 2^{33a} and HTLV-III^{33b} and to decrease the infectivity of *Treponema pallidum*.³⁴ Therefore, both clinical and laboratory evidence suggests that spermicides may protect against STD. Two randomized clinical trials, one community trial, one cross-sectional study, and four case-control studies have all shown a lower risk of STD among users. The consistency of these findings by different investigators, with different study populations,

Table V. Efficacy of oral antibiotic prophylaxis in preventing sexually transmitted diseases

Study	Study design	Study population	Regimen	Outcome	Measure of effect	95% Confidence interval
Eagle et al., 1949 ³⁹	Nonrandomized clinical trial	Naval crewmen	Penicillin, 100,000 units	Urethral gonorrhea	0.13	0.06-0.28
Regalla and Vilete, 1969 ⁴²	Nonrandomized clinical trial	Brazilian sailors	Doxycycline, 200 mg initially + 100 mg daily	Urethral gonorrhea, hard and soft chancre	0.13	0.05-0.36
Harrison et al., 1979 ⁴⁵	Randomized clinical trial	Naval crewmen, Far East	Minocycline, 200 mg	Urethral gonorrhea	0.46	0.30-0.72

and with different study designs suggests that this protective effect is real. However, most of these studies share the same methodologic flaws encountered in the studies of condom efficacy in preventing STD, and caution must be used in their interpretation as well.

Local measures. Several local prophylactic measures have been evaluated among persons at high risk of STD. These measures include vaginal application of antibiotics or antiseptics, prompt urination, and washing the genitals (Table IV). Daily use of vaginal Progonasyl,* a preparation of orthoiodobenzoic acid, among prostitutes lowered the rate of gonorrhea by 90%.³⁵ However, the study was flawed by administration of a curative dose of tetracycline to some of the women assigned to receive Progonasyl but to none of the controls. In addition, the possibility of sampling bias and the potentially confounding effect of number of contacts were not considered.

Prostitutes assigned to receive daily vaginal preparations of povidone-iodine for at least 18 months had a 60% lower risk of gonorrhea, a significant difference from placebo recipients.³⁶ However, the possibility of selection bias, ascertainment bias due to different frequencies of cultures and different rates of loss to follow-up, and confounding due to differences in sexual practices or self-medication were not considered.

To examine the effectiveness of urinating or washing the genitals in preventing gonorrhea in men, sailors were studied after having sexual contacts with prostitutes in the Far East.¹⁵ Neither washing the genitals within 1 hour nor urinating within 30 minutes had a statistically significant effect on acquisition of gonorrhea.

In another report,¹⁰ men who washed their genitals after coitus had an increased risk of gonorrhea. However, these studies did not consider the potential effects of selection bias and confounding due to different sexual practices.

Because of methodologic problems of these studies,

including failure to control for sexual activity, conclusions regarding the efficacy of local prophylaxis cannot be drawn.

Systemic antibiotic prophylaxis. In 1943, the Surgeon General of the United States Army authorized the military to use prophylactic oral sulfathiazole after a study³⁷ demonstrated its efficacy in reducing the gonorrhea rate among troops. After the emergence of sulfonamide-resistant strains of *Neisseria gonorrhoeae*, several placebo-controlled studies³⁸⁻⁴¹ conducted shortly after World War II showed that administration of oral penicillin within several hours after sexual intercourse reduced the rate of gonococcal infection in men. All of these studies had serious methodologic flaws, which included suboptimal randomization into treatment groups, poor compliance, failure to control for other prophylactic behaviors, and lack of information on exposure to infected partners. Only the study by Eagle et al.³⁹ contains sufficient data from which to calculate relative risk (Table V). Oral penicillin prophylaxis reduced the risk of gonorrhea by 90%, a significant difference from placebo controls. Penicillin prophylaxis never became widely used because of concern about selection of resistant strains and the risk of allergic reactions.

More recently, cohort studies were conducted with the use of analogues of tetracycline as prophylaxis for gonorrhea. Brazilian sailors on shore leave were given 200 mg of doxycycline orally, followed by 100 mg daily as long as sexual contact continued.⁴² Although antibiotic prophylaxis was highly effective in the prevention of symptomatic gonorrhea and hard and soft chancre, no cultures were obtained. Because asymptomatic infections would not have been detected, ascertainment bias may have occurred. In a double-blind placebo-controlled evaluation of prophylactic oral minocycline, 200 mg or placebo was administered under direct physician supervision to 1080 naval crewmen on shore leave in the Far East.⁴³ Later, at sea, gonococcal infection was detected in 5% of antibiotic recipients compared with 10% of placebo recipients, a highly significant difference. The incidence of infection with moderately resistant strains was reduced, but the incubation

*Use of trademark is for identification purposes only and does not imply endorsement by the Department of Health and Human Services.

period was nearly doubled among men who became infected. No increase in the proportion of asymptomatic infections was observed, and side effects were minimal. However, the authors feared that widespread use of prophylactic minocycline would be of limited value as a public health measure because of the tendency to select out resistant gonococci.

Acceptability of preventive measures

Restriction of sexual activities. Because it is difficult to identify and avoid infected sexual partners, limiting the number of partners may be the most effective "common sense" approach to reduce the risk of STD. Only minimal risk of infection occurs in a long-term monogamous relationship devoid of outside sexual encounters, namely, transmission of a preexistent sexually transmitted disease acquired by one partner before entering into the relationship. At the other extreme, much greater risk of acquiring infection occurs with sexual encounters with multiple anonymous partners who, in turn, have had multiple anonymous partners. Sexually active persons not in a monogamous relationship who are concerned about STD must be selective in the choice of sexual partners. A potential partner can be inspected for obvious lesions or discharge and questioned about the presence or likelihood of infection. In this era of viral infections for which treatments are palliative and not curative, frequent indiscriminate sexual activity is dangerous.

Refraining from sexual contact while infected is the most effective way to prevent transmission of infection. Persons undergoing treatment for STD are usually advised to avoid sexual activity with others until after completion of the therapeutic regimen. Those with active herpetic lesions are strongly advised to refrain from sexual activity until lesions have completely healed. Unfortunately, these persons often do not comply with such advice or are unaware of the risk of disease transmission.⁴⁴

Several studies show that some people will restrict their number of sexual partners and types of activities in response to perceived risk of STD. In a London study,⁴⁵ a group of hepatitis B carriers reduced their number of sexual contacts after receiving counseling regarding transmission of infection to sexual partners. Sixty percent of never-married respondents to a telephone survey who perceived themselves as being at risk for genital herpes had changed their behavior as a result of this concern.² Concern about acquired immune deficiency syndrome has dramatically influenced the sexual behavior of some homosexual men. Rates of rectal and pharyngeal gonorrhea in New York City declined dramatically in 1982,⁴⁶ and a similar decrease in gonorrhea incidence was reported among homosexual men attending a public clinic in Denver.⁴⁷ These de-

clines suggest some type of behavioral change. A large survey of homosexual men in San Francisco showed substantial reductions in bathhouse sexual activity and number of sexual partners between 1982 and 1983, together with a concurrent increase in monogamy.³ In another large survey of homosexual men in Chicago and Detroit, behavioral changes due to concern about acquired immune deficiency syndrome were reported by 91% and 79% of participants, respectively.⁴⁸ Of 54 homosexual men participating in a study of modifications in sexual practices after learning of the acquired immune deficiency syndrome epidemic, 83% had reduced their number of sexual partners, 74% had reduced their number of anonymous partners, 30% had increased their use of condoms, and 28% were more likely to insist that their partners use condoms.⁴⁹ These data clearly indicate that sexually active people are willing to change their sexual behavior to prevent the acquisition and transmission of STD.

Condom use. Condoms are inexpensive, available without a prescription, easy to use, and protective not only against STD but also against pregnancy. These features are advantageous for teenagers and young adults who account for most STD in the United States. To be effective, condoms must remain intact, and they can only protect the areas covered. A condom should be effective in protecting the wearer's partner from infectious agents that are transmitted in semen, for instance, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, hepatitis B, cytomegalovirus, and HTLV-III, as well as from those transmitted by direct penile contact. The condom should be able to protect the covered part of the user's genitals from warts, syphilis, chancroid, and infected vaginal secretions.

Men have been shown to object to condom use more than their female partners,⁵⁰ and they frequently cite impairment of pleasure as the reason.^{50,51} However, several large studies have demonstrated an acceptance of condoms by both men and women. In one,⁵¹ the majority of men and women attending an STD clinic claimed that they would not object to either using condoms or having their partners use condoms, and most claimed they would not be ashamed to ask for and buy condoms. Another large study⁵² involved distribution of free condoms to inner-city unmarried male adolescents through commercial outlets such as grocery stores and barber-shops. During the distribution period, use of a condom with last coitus steadily increased more than fourfold. Nearly equal numbers of recipients reported condom use for "protection against venereal disease" and to "prevent unwanted birth." Another frequently cited reason for condom use was "because the girls wanted me to." Perhaps women should assume a more assertive role in condom use, since it will decrease risk of pregnancy, most STD, and perhaps cervical cancer.

Current use of barrier contraception. Condom and diaphragm use increased dramatically between 1973 and 1982.⁵³ In 1982, one third of married women using contraception to delay pregnancy were using condoms or diaphragms, compared with only 16% in 1973. Condom use rose from 12% to 18%, and diaphragm use from 4% to 15%. Of married women using nonsurgical contraception, 24% were using the condom in 1982. Of sexually active never-married women aged 15 to 19 who were using contraception, 28% were using the condom (22%) or diaphragm (6%).

Spermicide use. Like condoms, vaginal spermicides are inexpensive, available without a prescription, easy to use, and protective against pregnancy as well as STD. Data regarding the use of vaginal spermicides are limited. Vaginal spermicides are safe and are unlikely to cause systemic side effects.^{54, 55} It is unknown whether anorectal applications of spermicides will reduce the risk of anally acquired STD, and their effects on rectal mucosa have not yet been tested.

Use of systemic antibiotics. While prophylactic oral systemic antibiotics may be effective in reducing the risk of STD, their widespread use cannot be recommended for several reasons: (1) No single agent protects against all STD; (2) medical supervision is necessary; (3) they carry the risk of allergic reactions and side effects; (4) most important, they may lead to emergence of resistant strains of organisms.

Impact of prophylaxis

The public health impact of personal prophylaxis against STD may be large, and the effect on STD incidence far exceeds that due to direct prevention of individual cases. As with any communicable disease, prevention of one case results in the prevention of many subsequent cases. Several attempts to mathematically model this effect⁵⁶⁻⁵⁸ for gonorrhea have all shown that gonorrhea incidence is exquisitely sensitive to changes in probability of transmission. Using a basic model for heterosexuals developed by Hethcote and Yorke,⁵⁶ which employs several simplifying assumptions about prevalence, sexual activity, and disease duration, we calculated that reducing the probability of transmission of gonorrhea by 10% per partner would reduce the prevalence of gonorrhea in the heterosexual population by more than 50%. The recent resurgence in use of barrier methods of contraception, especially among younger couples,⁵⁹ may be responsible in part for recent declines in gonorrhea rates nationwide.⁶⁰

In conclusion, some sexually active persons either are willing to change or else have already changed their sexual behavior in response to perceived risk of STD. Mechanical barriers and spermicides are safe, effective, and acceptable preventive measures. Clinicians should advocate use of barriers among those at high risk for

STD, regardless of use of other methods of contraception, such as sterilization or oral contraceptives. In summary, the following measures appear reasonable in reducing the risk of acquisition or transmission of STD:

1. Limit the number of sexual partners.
2. Avoid sexual encounters with anonymous persons or those with multiple sex partners.
3. Avoid sexual contact with infected persons or those undergoing treatment for STD.
4. Inspect the genitals of potential sex partners for lesions or discharge; ask them if they have STD.
5. Use barriers and spermicides as disease prophylactics, regardless of need for contraception.
6. While infected with STD, refrain from sexual activity until treatment has been completed and symptoms have subsided.

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Initiation of oogenesis in the human fetal ovary: Ultrastructural and squash preparation study

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Ovaries from 27 human fetuses at ≤ 12 weeks' gestational age were examined with electron microscopy and squash preparation studies. The findings demonstrated that meiosis begins in the fetal ovary between 11 and 12 weeks of age. This is several weeks after gonadal sex differentiation, which can be recognized in the testis at 6 weeks. Ovaries at earlier ages included primitive germ cells and oogonia. With advancing fetal age, mitotic activity and germ cell degeneration became increasingly evident. Preleptotene cells were found as early as 9 to 10 weeks, suggesting that meiotic capacity is present soon after gonadal sex differentiation but further progression into meiosis is delayed until some specific inductive influence is exerted. (AM J OBSTET GYNECOL 1986;155:189-95.)

Key words: Fetal ovary, oogenesis, meiosis

Oogenesis in humans, as in other mammals, begins early in development. Germ cells enter and proceed through the first meiotic prophase in the fetal and early neonatal period, with the definitive population of oocytes becoming incorporated in follicles during this time. The general pattern of oocyte differentiation in the human fetus has been described in several reports, including histologic, ultrastructural, and chromosomal observations.¹⁻⁵

While such studies have provided important information on the changes associated with oocyte and follicle formation in the human fetal ovary, a clear picture of the early changes related to the initiation of meiosis is not evident. For example, among the most extensive and frequently cited studies, the time of onset of meiosis is variously indicated as 2 months,¹ 10 weeks,² 12 weeks,³ and 13 weeks.⁴ In some cases, the discrepancy can be attributed to using menstrual history rather than fetal measurements. However, additional problems are related to the relatively small number of specimens from the earlier ages, inadequate cellular preservation, and technical limitations, such as the lack of ultrastructural correlation.

Table I. Number of human fetal ovaries examined at different ages and general appearance of germ cells

Age (wk)	Foot length (mm)	No.	Appearance
6-8	3-5	9	Scattered primitive germ cells, undifferentiated appearance
8-9	5-6.5	3	Primitive germ cells concentrated in differentiating cortex
9-10	6.5-8	7	Oogonia with occasional mitoses, scattered degenerating cells
10-11	8-9.5	4	Oogonia in groups, many mitoses, degenerating cells
11-12	9.5-11	4	Active mitosis, increased degeneration, few cells in meiosis

To enable better understanding of the potential effects of exogenous agents on developing oocytes as well as intrinsic chromosomal disorders, it is important to define precisely the time of onset of oogenesis. Furthermore, awareness of the cellular changes immediately preceding and accompanying the initiation of meiosis is of particular interest, since it is during this period that critical genetic events affecting subsequent developmental and reproductive capabilities occur.

We have previously described the ultrastructural changes associated with oocyte differentiation in the human fetus.⁵ However, the material for that study included very few specimens from the earlier ages. More

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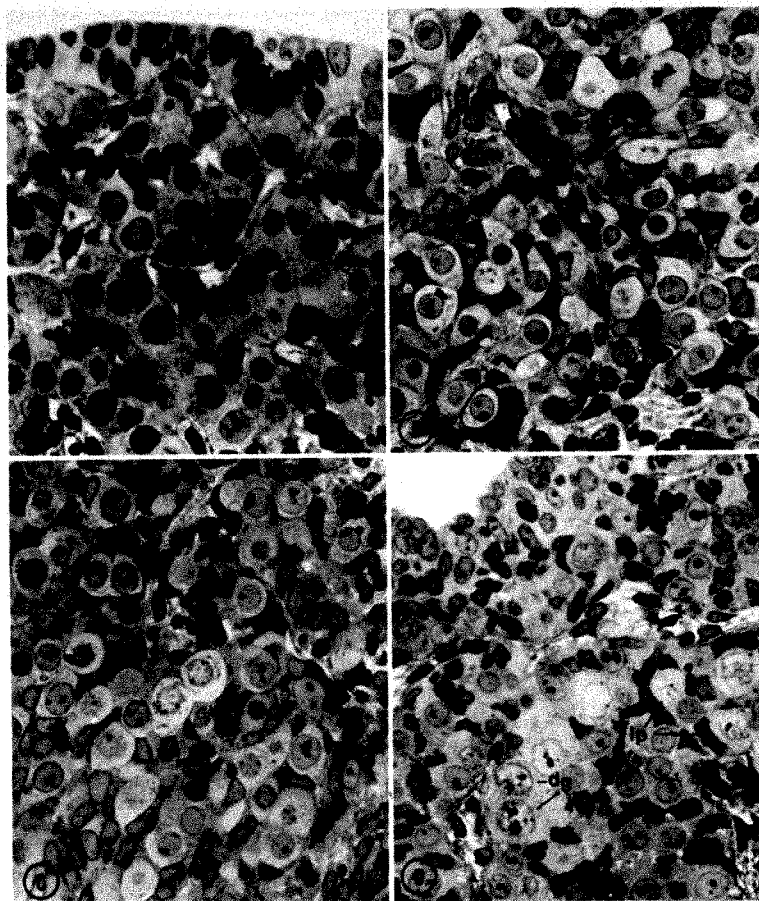


Fig. 1. Light micrographs of human fetal ovary. *A*, 9 weeks' gestation. *B*, Oogonia with prominent nucleoli, 9½ weeks. *Arrow* indicates mitosis. *C*, Oogonia are numerous at 10½ weeks. Note cells with chromatin condensation (*arrows*). *D*, Leptotene cells (*lp*) and degenerating germ cells (*dg*), 12 weeks' gestation. (Toluidine blue. Original magnification $\times 400$.)

recently, we have had the opportunity to examine a series of ovaries from 27 human fetuses at ≤ 12 weeks' gestational age. The specimens were studied with electron microscopy and squash preparations to indicate the cellular and chromosomal features at the time that oogenesis begins.

Material and methods

Fetal gonadal specimens were obtained after abortion by suction extraction. Fetal age was determined by foot length.⁶ In those fetuses in which intact gonads could be identified, the specimens were dissected from the abdominal cavity with the aid of a dissecting microscope. Gonadal tissue was divided into specimens placed directly into Bouin's solution, glutaraldehyde, alcohol-acetic acid, and minimal essential medium. Delay in tissue preservation was minimal, since the individual performing the abortion (L. W.) also did the gonadal dissection. The protocol for the investigation was approved by the Medical Research Ethics Committee, Copenhagen.

Specimens were initially examined by light microscopy to confirm the presence of gonadal elements, determine general adequacy of cell preservation, and indicate gonadal sex. Specimens were identified as testes by the presence of seminiferous cord formation. All other specimens were considered ovaries by exclusion in accordance with established standards for identifying gonadal sex in the age group studied.³ Among the ovarian specimens, 27 were considered to have adequate cellular preservation for further evaluation.

Tissue fixed for electron microscopy was embedded in Epon, sectioned with a Reichert Ultracut ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Philips 201 electron microscope. Semithin sections were stained with toluidine blue for light microscopic evaluation.

Squash preparations for chromosomal analysis were performed as previously described.⁷ Briefly, after fixation in 3:1 ethyl alcohol/glacial acetic acid at 0° C for 24 hours, a small portion of tissue was placed in a drop of freshly prepared 45% glacial acetic acid on a sili-

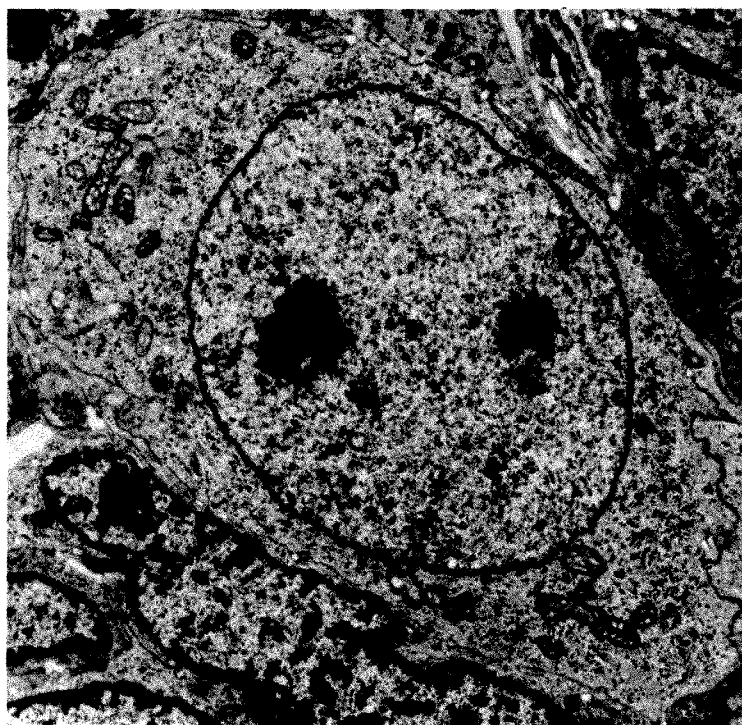


Fig. 2. Electron micrograph of primitive germ cell, 9 weeks' gestation with eccentric nucleus and abundant glycogen. (Original magnification $\times 8500$.)

conized coverslip. A precleaned gelatinized slide was then placed face down on the coverslip with evenly applied pressure. The slide was then placed flat on a block of dry ice and after a few minutes the coverslip was flipped off with the use of a razor blade and the slide immersed in 95% ethyl alcohol. After removal from the alcohol, the slide was air-dried and stained with a freshly prepared Giemsa staining solution. Slides were examined and photographed with a Zeiss Ultraphot III microscope.

Results

The numbers of specimens at different ages are indicated in Table I, which also summarizes the general appearance of the germ cells as seen by light microscopy. Illustrations of the histologic appearance are shown in Fig. 1. As indicated in the table, the earliest specimens were essentially undifferentiated and were classified as ovaries because of the absence of testicular cord formation. The germ cells were large and irregular in contour, characteristic of primitive germ cells. By 8 to 9 weeks, a tendency of the germ cells to concentrate peripherally was associated with a beginning, although still indistinct separation of cortex and medulla. At 9 to 10 weeks, the germ cells assumed a more rounded contour typical of oogonia. A distinct increase in mitotic activity was evident. There was a further increase at 10 to 11 weeks, as groups of dividing oogonia were often seen. At this time, there were many

degenerating cells and because of the prominent chromatin condensation it was difficult to distinguish degenerative and possible early meiotic changes. Specimens from 11 to 12 weeks also showed many mitotic and degenerating germ cells. In addition, a few cells present in the inner cortex had nuclear features consistent with oocytes in early meiosis.

By electron microscopy, the primitive germ cells present in the earliest specimens were characterized by irregular ovoid shapes with pseudopodia-like surface projections (Fig. 2). Nuclei contained evenly distributed chromatin and prominent nucleoli. Chromatin condensation associated with degenerative changes was not seen in these cells and there was infrequent mitotic activity.

The appearance of oogonia, germ cells with a smooth, regular contour and generally uniform structure (Fig. 3), was first seen between 9 and 10 weeks. At this time, there was an evident increase in germ cell number and a tendency for the cells to be arranged in groups. Nucleoli initially were relatively small but later increased in size and complexity. The pattern of chromatin distribution also changed, with many of the cells at 10 to 11 weeks exhibiting chromatin condensation. By electron microscopy, it was evident that the chromatin condensation was the result of dense, irregular chromatin aggregation (Fig. 4). This finding together with extensive cytoplasmic vesiculation indicated degenerative rather than early meiotic changes. The pres-

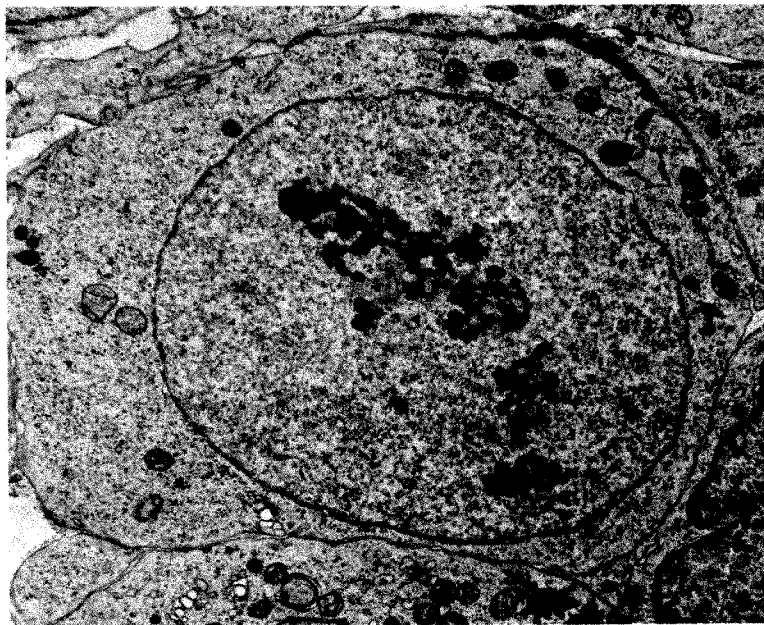


Fig. 3. Oogonium at 10 weeks shows elaborate nucleolus. (Original magnification $\times 8500$.)

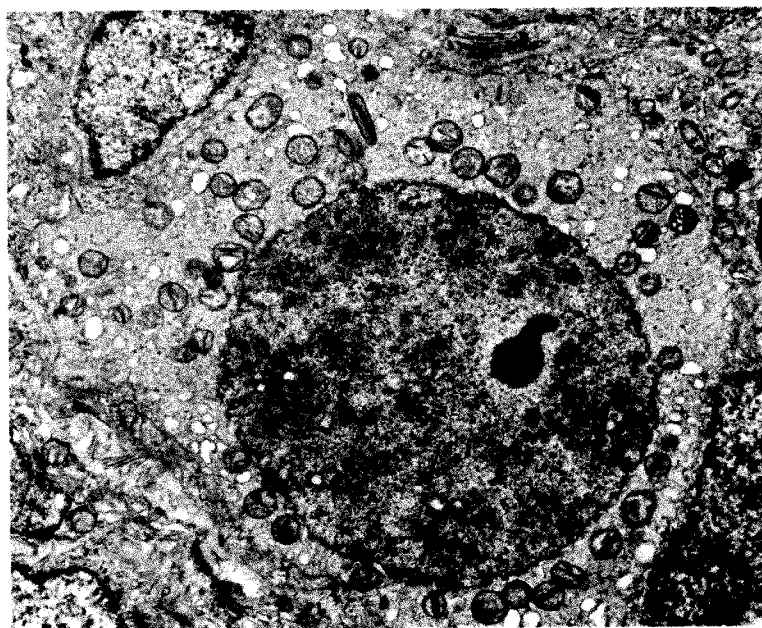


Fig. 4. Oogonium with dense, irregular chromatin condensation and numerous cytoplasmic vesicles, 10½ weeks. (Original magnification $\times 8000$.)

ence of threadlike structures characteristic of early meiotic cell nuclei was not observed in these specimens. Perinuclear arrangement of mitochondria, a feature of human oocyte differentiation, also was not seen except for rare scattered cells.

The latter changes were clearly evident in the inner cortex at 11 to 12 weeks, indicating the differentiation of oocytes in leptotene (Fig. 5). Pairing of threadlike structures was seen in some of the cells. There were

also many cells in mitosis and undergoing degeneration at this time. The different cellular changes were readily distinguishable at the electron microscopic level.

The squash preparations provided additional information on the chromosomal patterns in the germ cells. Up to 9 weeks, only interphase and occasional mitotic configurations were seen. Beginning at 9 to 10 weeks and increasing at 10 to 11 weeks, cells with dense chromosome masses and a delicate network of filaments

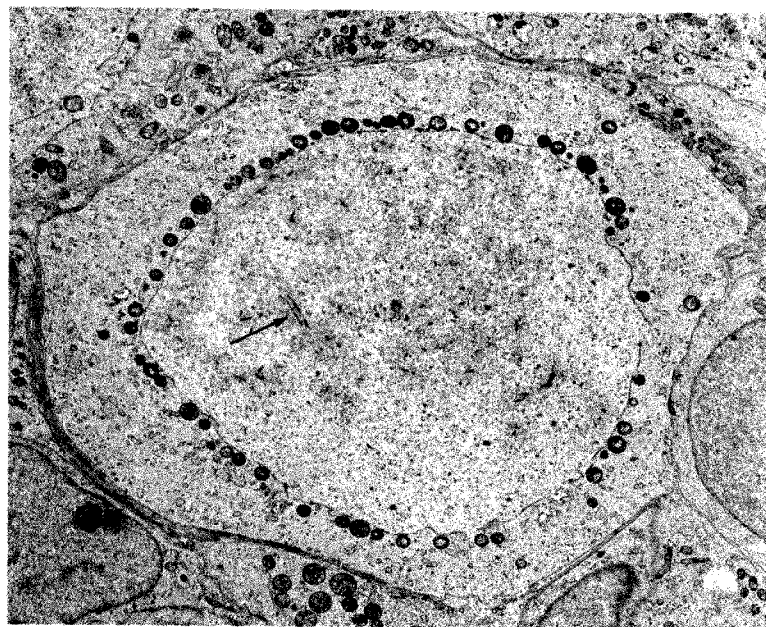


Fig. 5. Oocyte in meiosis, 12 weeks' gestation. Note synaptonemal complex (arrow) and perinuclear distribution of mitochondria. (Original magnification $\times 6500$.)

characteristic of preleptotene cells were identified (Fig. 6, A). Various stages of the condensation process could be seen (Fig. 6, B). Leptotene configurations were not present at these ages but were identified at 11 to 12 weeks, along with preleptotene, mitotic, and interphase figures. The leptotene cells were characterized by densely arranged chromosomal filaments with small beaded areas of condensation. (Fig. 6, C).

Comment

The present findings, with the use of ultrastructural and chromosomal analysis, indicate that meiosis begins in the human fetal ovary between 11 and 12 weeks' gestational age. This is in agreement with the findings of van Wagenen and Simpson,³ based on extensive light microscopic observations. The earliest specimen in which they found cells in meiosis was from a 12-week-old fetus. Manotaya and Potter¹ indicated 13 weeks as the time of onset of meiosis, but they used menstrual age and therefore the findings are comparable. The reason for the discrepancy in those reports^{1,2} describing an earlier age of onset is not clear but could be related to difficulty in determining fetal age since crown-rump measurements were not given for the specimens with meiosis.

In estimating fetal age, we were unable to use crown-rump length or other recommended indices because of the nature of the abortion procedure, which often resulted in disruption of anatomic relationships. However, foot length could always be determined and this has been found to be a reliable method for estimating fetal age.⁶ Comparisons made with specimens obtained

in our previous study in which accurate crown-rump measurements were available indicated good correlation in histologic and ultrastructural appearance at comparable estimated ages. The general consistency in appearance and stage of differentiation of ovaries from fetuses with similar foot length measurements also supports the reliability of this method.

The onset of meiosis at the end of the third month indicates that there is considerable delay between the time of gonadal sex differentiation and initiation of oogenesis. Testicular differentiation can be recognized more than a month earlier, at 6 to 7 weeks.² It has previously been suggested that important species differences in the pattern of structural and endocrine differentiation of the female reproductive system in mammals might relate to the existence of two separate groups: those with onset of meiosis immediately after gonadal sex differentiation, such as the mouse, rat, and hamster, and those with delayed meiosis, such as the rabbit and cow.⁸ The present findings would indicate that the human falls in the latter group.

However, it is of interest that preleptotene cells can be identified as early as 9 to 10 weeks, that is, relatively soon after gonadal sex differentiation. Preleptotene cells are difficult to identify by light microscopy because of some similarity in appearance to degenerating cells based on chromatin condensation. Even with electron microscopy there are no specific criteria for classifying cells as preleptotene, since the chromosomal pattern associated with this stage of differentiation is not clearly demonstrated by the methods used for ultrastructural studies. Therefore studies with the use of squash prep-

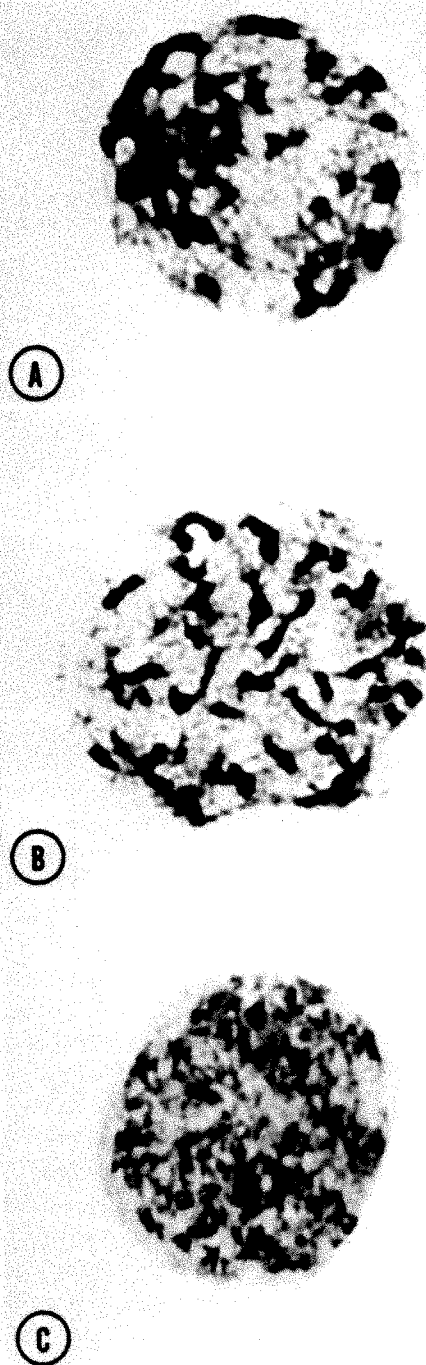


Fig. 6. Squash preparations, fetal ovarian germ cells. A, Preleptotene cell, 9½ weeks' gestation. B, Complete condensation, with dense chromosome masses and fine filamentous threads, 10½ weeks. C, Leptotene oocyte, 12 weeks' gestation. (Original magnification $\times 1200$.)

arations or comparable methods for chromosome analysis are the only reliable way of identifying such cells.

Our finding of preleptotene cells in the human fetal ovary as much as 2 weeks before the appearance of later stages of meiosis raises some question as to their significance. It is possible, as some have suggested,⁹ that

the deoxyribonucleic acid synthesis associated with preleptotene differentiation is a postmitotic rather than a premeiotic stage. Whether this is more than a semantic distinction remains unclear at the present time. A possible explanation for the early appearance of preleptotene cells in the human fetal ovary is that these cells do indeed represent an early meiotic stage but further progression into meiosis is delayed until some specific inductive influence is exerted. Previous studies have demonstrated the presence of a meiosis-inducing substance in gonadal tissue of various mammalian species, including the human.^{8, 10} The precise time of appearance and mechanism of action of meiosis-inducing substance remain to be determined. The present findings would be consistent with an effect beginning sometime after the initial appearance of preleptotene cells, perhaps requiring a preliminary priming period. Further studies would be needed to test this idea.

It should be stressed that careful correlation of different techniques and attention to methods of cell preservation and processing are essential in studies on early germ cell differentiation. A particularly difficult problem is presented by the large number of degenerating cells present in the fetal ovary. They can easily be misinterpreted as cells in mitosis or meiosis. Previous reports describing the cellular changes in the human fetal ovary have indicated that germ cell degeneration is a widespread phenomenon, well over half the peak number of germ cells degenerating during fetal development.^{1, 11} The importance of this phenomenon relates not only to its biologic implications but also to problems in morphologic evaluation.

In summary, initiation of oogenesis, as indicated by ultrastructural and squash preparation studies, occurs between 11 and 12 weeks in the human fetal ovary. Leptotene cells representing the earliest stage of meiotic prophase first appear at this time. Ovaries from fetuses at younger ages contain numerous preleptotene cells, suggesting that meiotic capacity is present as early as 9 to 10 weeks. Further studies are needed to clarify the mechanisms responsible for the progression from preleptotene to leptotene associated with the onset of meiosis.

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Estrogen effects on plasma volume, arterial blood pressure, interstitial space, plasma proteins, and blood viscosity in sheep

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In adult castrated ewes the infusion of 17 β -estradiol for 3 weeks was associated with a 12% increase in body weight, a 20% increase in whole blood volume (mainly due to a 27% increase of plasma volume), a 13% decrease in mean arterial blood pressure, and a 40% increase in heart rate. The change in plasma volume correlated with the change in estradiol concentration ($r = 0.72$). Most of the fluid was retained in the interstitial space, as represented by a 6 kg weight gain, 10% of which was in the intravascular compartment. Whole blood and plasma viscosity increased 16% and 21%, respectively, thus reversing some of the blood volume effects toward a hyperdynamic cardiovascular state. We conclude that many of the cardiovascular and hematologic changes with estrogen administration are similar to the changes observed during pregnancy, with the proposed requirement of decrease in mean arterial blood pressure as a condition for blood volume expansion. (*AM J OBSTET GYNECOL* 1986;155:195-201.)

Key words: Estrogen, blood volume, blood pressure, pregnancy

Human pregnancy is characterized by a 35% to 45% increase in blood volume from the nonpregnant values, mainly secondary to a 45% to 60% increase in plasma volume and a 20% to 30% increase in red cell mass.¹ Among other cardiovascular changes, diastolic arterial pressure decreases about 10 to 15 mm Hg and heart rate increases 10 to 15 bpm.² Also, plasma protein concentrations alter to affect both the Starling forces, and thus fluid flux across the capillaries, and blood viscosity, which is a major determinant of vascular resistance.³

During human pregnancy the concentrations of several hormones that play a role in fluid regulation increase in maternal plasma, including progesterone, de-

oxycorticosterone, aldosterone, and prolactin. A large increase also occurs in the concentration of 17 β -estradiol and other estrogens. Experimentally it has been shown that estrogen administration to postmenopausal women, men with prostate cancer, and laboratory animals results in blood volume increases secondary to plasma volume expansion.⁴

In an effort to test the hypothesis that some of the cardiovascular and hematologic changes of pregnancy, such as the increase in plasma and whole blood volumes, heart rate, and decrease in mean arterial blood pressure, are a function of the circulating estrogen concentration, we infused 17 β -estradiol into castrated ewes for 3 weeks and measured whole blood, plasma, and red cell volumes, arterial pressure, heart rate, plasma protein concentrations, and plasma and whole blood viscosities. The studies were not meant to reproduce exactly the endocrinologic milieu of pregnancy.

Material and methods

Five adult ewes were used in the present study. Four to 7 days after they were received from a local breeder,

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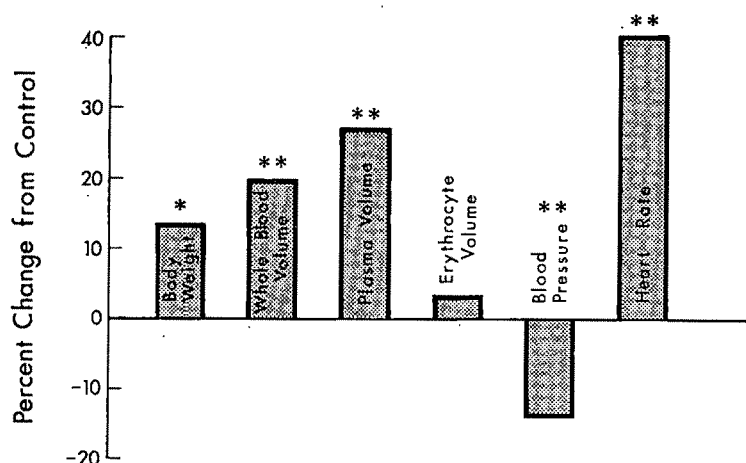


Fig. 1. Percent change from control values for body weight, whole blood, plasma, and erythrocyte volumes, mean arterial blood pressure, and heart rate. * = $p < 0.05$; ** = $p < 0.01$.

they were oophorectomized under spinal anesthesia. We placed Tygon catheters in a hindlimb artery and vein and exteriorized them to a flank pouch. Subsequently we flushed the catheters daily with 5 ml of heparinized saline solution (50 IU of heparin per milliliter) and allowed the animals to recover from the surgical stress for 3 weeks. Then we placed the ewe in a special metabolism cart and for 3 weeks monitored their body weight, arterial blood pressure, heart rate, and blood volumes. We then continuously infused 17β -estradiol (Sigma Chemical Co., St. Louis) at the rate of either 6.7 or 33.6 mg/kg/day for 3 weeks. Three ewes were infused in a random manner with both doses with a 4-week interval between infusions, during which time the various functions were measured. Of the other two ewes, one was infused with the lower dose, while the other received the higher dose. Thus four ewes were infused at each dose. In addition, three ewes were infused at the rate of 3.3 mg/kg/day, but no significant changes were observed in any of the parameters measured. In essence this constituted a control experiment for infusion of the vehicle. The 17β -estradiol was dissolved in ethanol and then diluted in normal saline solution. The rate of saline infusion was 4 ml/hr, for a total of 96 ml/day; the concentration of ethanol in saline solution was 0.1%. This minimized the effects of both saline volume and ethanol on the ewe's cardiovascular system. Then at weekly intervals we determined arterial blood pressure, heart rate, plasma, erythrocyte, and whole blood volumes, blood plasma protein concentrations, and plasma viscosity.

Blood volume measurements. We determined the total blood volume by dilution of chromium 51-labeled red cells, and calculated the plasma volume indirectly from the whole blood volume and the microhematocrit.⁵ The day of the experiment we labeled 3 ml blood with ^{51}Cr using standard techniques.⁵ We obtained a 0.5 ml blood sample for background radioactivity and then

injected the tagged erythrocytes through the venous catheter, which was flushed three times the catheter volume with heparinized saline solution. Thereafter we withdrew 0.5 ml blood samples at 10, 20, and 30 minutes through the arterial catheter. To clear the catheter, we withdrew a volume equal to six times that of the catheter before each sample was obtained and then replaced that blood. We measured the sample radioactivity in a gamma counter (Packard Instrument Co., Downers Grove, Illinois) and corrected it for hematocrit and sample weight. Then by the use of a computer program, we extrapolated the decay curve back to zero time and used this value to calculate whole blood volume. Whole blood, plasma, and erythrocyte volumes were expressed both in absolute values and per weight of the ewe.

Arterial pressure and heart rate. Arterial blood pressure was recorded with a pressure transducer (Gould, P23Db, Cleveland) on a polygraph (Gould, Model 200), which was connected to an on-line computer (Texas Instrument, 990/10, Houston). Following a 1-hour period of adaptation, the mean arterial pressure and heart rate were obtained for an additional hour, averaged over 1-minute intervals, and stored for later analysis.

Plasma protein determinations. At weekly intervals we withdrew arterial blood into a sterile, nonheparinized plastic syringe and immediately transferred it into an appropriate glass tube. We determined fibrinogen concentration by a timed conversion of fibrinogen to fibrin by topical thrombin, using a fibrometer (Fibrosystem, Cockeysville, Maryland) to detect clot formation. Each time we constructed a standard curve, using dilutions of a commercial plasma of known concentration (Data-Fi Fibrinogen Calibration Reference, Dade Diagnostics Inc., Aguada, Puerto Rico).

Total plasma protein and albumin concentrations were determined by the biuret method⁶ and a modified bromocresol green binding method,⁷ respectively. The

Table I. Cardiovascular and hematologic values in ewes before and after 3 weeks infusion with 17 β estradiol*

Variable	Control	Change	% change
Body weight (kg)	55.11 $\pm$ 2.14	6.44 $\dagger$ $\pm$ 1.83	11.7 $\dagger$
Whole blood volume (ml)	3,355 $\pm$ 124.5	653.8 $\dagger$ $\pm$ 125.9	19.5 $\dagger$
Plasma volume (ml)	2,346 $\pm$ 94.2	627.5 $\dagger$ $\pm$ 117.9	26.7 $\dagger$
Erythrocyte volume (ml)	1,009 $\pm$ 62.2	17.9 $\pm$ 57.6	1.8
Whole blood volume (ml/kg)	61.1 $\pm$ 1.4	4.26 $\dagger$ $\pm$ 1.03	7.0 $\dagger$
Plasma volume (ml/kg)	42.8 $\pm$ 1.7	5.58 $\dagger$ $\pm$ 2.12	13.0 $\dagger$
Erythrocyte volume (ml/kg)	18.2 $\pm$ 0.5	-1.48 $\pm$ 0.66	-8.1
Hemoglobin (gm/dl)	12.0 $\pm$ 0.6	-0.22 $\pm$ 0.39	-1.8
Hematocrit (%)	30.0 $\pm$ 1.3	-0.97 $\pm$ 1.04	-3.2
Blood pressure (mm Hg)	90.9 $\pm$ 1.9	-12.0 $\dagger$ $\pm$ 2.9	-13.2 $\dagger$
Heart rate (bpm)	65.3 $\pm$ 5.0	26.3 $\dagger$ $\pm$ 6.0	40.3 $\dagger$
Estrone (pg/ml)	4.59 $\pm$ 1.20	155.8 $\dagger$ $\pm$ 130.2	3,394 $\dagger$
17 β -Estradiol (pg/ml)	20.0 $\pm$ 8.8	6,242 $\dagger$ $\pm$ 1,103	31,210 $\dagger$

*Mean $\pm$ SEM, n = 8.

$\dagger$ p < 0.05.

$\ddagger$ p < 0.01.

plasma globulin was determined by the difference of these values.

Blood viscosity determinations. We measured the absolute viscosity of distilled water, whole blood, and plasma at 37° C in centipoise, using a Coulter capillary viscometer (Coulter Electronics Ltd., Hialeah, Florida). Viscosity was calculated relative to distilled water.

Estrogen assays. Unconjugated unbound and bound serum estrone and 17 β -estradiol were measured by radioimmunoassay by means of a modification of the method of Abraham et al.⁸ (Radioassay Systems, Carson, California). The intra-assay and interassay coefficients of variation were 7% and 13%, respectively, for both hormones.

Data analysis. From the control and experimental measurements as well as paired differences between experimental and control values, we computed mean, SD, and SE of the means for each variable under consideration. We tested differences between means by a paired *t* test at the 0.05 and 0.01 levels.

Results

Body weight. During the 3 weeks before estradiol infusion there was no significant change in body weight or other parameter. In contrast, during the 3 weeks of estradiol infusion the ewe's body weight increased about 12% (6.4 $\pm$ 1.8 kg, mean $\pm$ SEM) from 55.1 $\pm$ 2.1 kg (p < 0.05) at both 17 β -estradiol doses (Fig. 1 and Table I).

Blood volume. Fig. 1 also shows the changes of whole blood volume, which gradually increased about 19% (654 $\pm$ 126 ml) at 3 weeks, from 3355 $\pm$ 125 ml at both 17 β -estradiol doses (p < 0.01). When calculated per kilogram of body weight, whole blood volume increased about 7% from a control value of 61.1 $\pm$ 1.4 ml/kg (p < 0.05) (Table I).

Essentially all of the whole blood volume increase could be accounted for by the rise in plasma volume,

which by the end of 3 weeks increased about 27% (628 $\pm$ 118 ml) from 2346 $\pm$ 94 ml (p < 0.01). With the lower dose the rate of increase was gradual over the entire 3 weeks, while at the higher dose the increase was relatively rapid, occurring in 1 to 2 weeks. Again, when normalized to body weight, plasma volume increased 13% (5.6 $\pm$ 2.1 ml/kg) from a control value of 42.8 $\pm$ 1.7 ml/kg (p < 0.05) (Table I). The whole blood and plasma volume changes returned to control values by two weeks after infusion. Erythrocyte volumes did not change significantly from the control values of 1009 $\pm$ 62 and 18.2 $\pm$ 0.5 ml/kg (Table I).

Estrogen concentration. The plasma concentrations of 17 β -estradiol and estrone demonstrated very large increases (Table I). The mean plasma estradiol concentrations were 174 $\pm$ 17, 4616 $\pm$ 616, and 6991 $\pm$ 875 pg/ml at the infusion rates of 3.3, 6.7, and 33.6 mg/kg/day, respectively. For estrone these values were 6.5 $\pm$ 2, 179.6 $\pm$ 21.2, and 239.3 $\pm$ 87 pg/ml, respectively. As shown in Fig. 2, the change in plasma volume was highly correlated with the change in 17 β -estradiol concentration (r = 0.72, p < 0.001).

Arterial blood pressure and heart rate. The mean arterial blood pressure decreased 13% (12 $\pm$ 2.9 mm Hg) from 90.9 $\pm$ 1.9 mm Hg (p < 0.01) following the 3-week 17 β -estradiol infusion. In turn, the heart rate increased about 40% (26 $\pm$ 6 bpm) from a control value of 65 $\pm$ 5 bpm (p < 0.01) (Table I and Fig. 1).

Blood plasma proteins and viscosity. As shown in Fig. 3 and Table II, plasma fibrinogen concentration increased 86% (148 $\pm$ 54 mg/dl) from 173 $\pm$ 15 mg/dl. The plasma albumin concentration decreased 7% from 3.7 $\pm$ 0.8 gm/dl, while the globulin increased 24% from 3.9 $\pm$ 0.4 gm/dl. The albumin-to-globulin ratio decreased 31% from 0.99 $\pm$ 0.13 (Fig. 3).

Plasma viscosity increased 21% from 1.58 $\pm$ 0.17 centipoise (p < 0.01), while whole blood viscosity increased 16% from 3.05 $\pm$ 0.30 centipoise (p < 0.01)

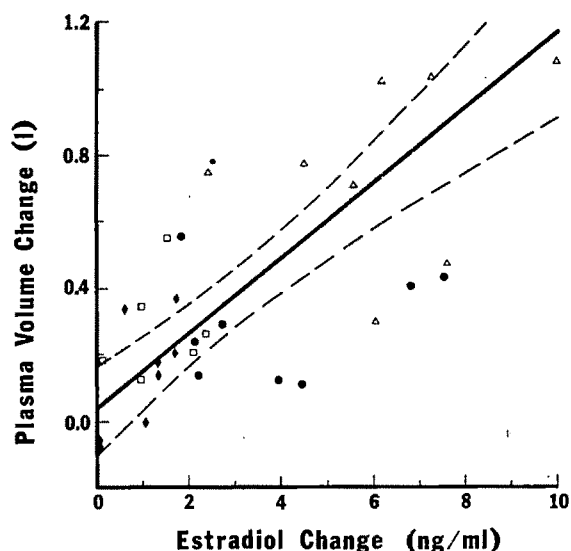


Fig. 2. Change in plasma volume (L) as a function of increase in circulating 17β -estradiol concentrations (ng/ml) following 2 and 3 weeks of estradiol infusion at 3.3 mg/kg/day ($\square$), $n = 6$; 6.7 mg/kg/day ($\bullet$), $n = 8$; and 33.6 mg/kg/day (Δ), $n = 8$. $\diamond$ represents the recovery values 3 weeks following the infusion of the two higher doses (i.e., 6.7 and 33.6 mg/kg/day, $n = 8$).

(Table II and Fig. 3). As observed earlier, in three sheep infused with 17β -estradiol at 3.3 mg/kg/day, no significant changes occurred in any of the parameters measured.

Comment

Hemodynamics. The first aim of this study was to test the hypothesis that profound hematologic and cardiovascular changes, such as occur during pregnancy, can be secondary to elevated circulating estrogen concentrations. Human pregnancy is associated with some rather striking cardiovascular adaptations, such as 40% and 55% increases in whole blood and plasma volumes, respectively, several liters of fluid accumulation in the interstitial space, a 10% increase in heart rate, 30% increase in cardiac output, and decreases in both systemic vascular resistance and mean arterial blood pressure.^{1,9}

Basically two theories have been postulated to explain these pregnancy-associated changes. Burwell et al.^{10,11} in the late 1930s first postulated that the placental circulation acts as an arteriovenous shunt. In experimental animals the opening of a shunt produces an immediate fall in vascular resistance, which in turn results in a drop in arterial pressure and cardiac output. Within a few seconds vasoconstriction results in compensatory blood flow redistribution. Heart rate increases, as does cardiac output, while arterial pressure gradually returns toward normal values. The decrease of renal perfusion, acting probably through the renin-angiotensin-aldosterone system, results in fluid reten-

Table II. Plasma protein and viscosity changes in ewes before and after 3 weeks' infusion of 17β -estradiol*

	Control	Change	% change
Fibrinogen (mg/dl)	173 $\pm$ 14.8	148 $\pm$ 54.0†	85.5†
Albumin (gm/dl)	3.67 $\pm$ 0.75	-0.25 $\pm$ 0.09‡	-6.8‡
Globulin (gm/dl)	3.85 $\pm$ 0.41	0.92 $\pm$ 0.02†	23.9†
Albumin/globulin ratio	0.99 $\pm$ 0.13	-0.31 $\pm$ 0.07†	-31.3†
Whole blood viscosity (centipoise)	3.05 $\pm$ 0.30	0.50 $\pm$ 0.17†	16.4†
Plasma viscosity (centipoise)	1.58 $\pm$ 0.17	0.33 $\pm$ 0.12†	20.9†

*Mean value $\pm$ SEM, $n = 8$.

† $p < 0.01$.

‡ $p < 0.05$.

tion with blood volume expansion within a matter of minutes to hours.^{12,13}

The second theory proposes that the hormonal changes during pregnancy, chiefly large increases in circulating estrogens, increase blood volume, cardiac output, and uteroplacental blood flow.⁴ Again, the mechanism probably involves estrogenic mediated stimulation of the renin-angiotensin system, producing an increase in aldosterone, with resultant sodium and fluid retention,¹⁴ as well as direct action on the uteroplacental vessels.^{15,16} Estrogen administration also has been shown to decrease the number of angiotensin receptors¹⁷ and to increase the amount of vasodilatory prostaglandins.¹⁸ In pregnant women aldosterone secretion increases markedly, reaching a peak near the end of gestation.¹⁹

The infusion of either angiotensin II²⁰ or aldosterone²¹ into experimental animals on a long-term basis has been shown to result in transitory fluid retention. However, the mean arterial pressure increase in turn results in increased glomerular filtration rate, so that blood volume returned to a normal value. Saline infusion demonstrates a similar effect.²² Only when renal arterial pressure is maintained within normal levels with a servomechanism as control is it possible to keep the fluid volume persistently elevated.^{20,21,23} Thus it appears that a prerequisite to maintaining an expanded blood volume is to maintain a relative or absolute decrease in renal arterial pressure.

During the course of gestation in sheep, whole blood and plasma volumes normally increase only about 6% and 8%, respectively.²⁴ Whole blood and plasma volumes in sheep with twins and triplets increase 9% and 12%, respectively (Ueda et al., unpublished observations). Circulating estrogen concentrations are comparably low in this species, rising from 20 to 400 pg/ml only during the last few days of gestation.²⁵

The present findings that in castrated sheep the

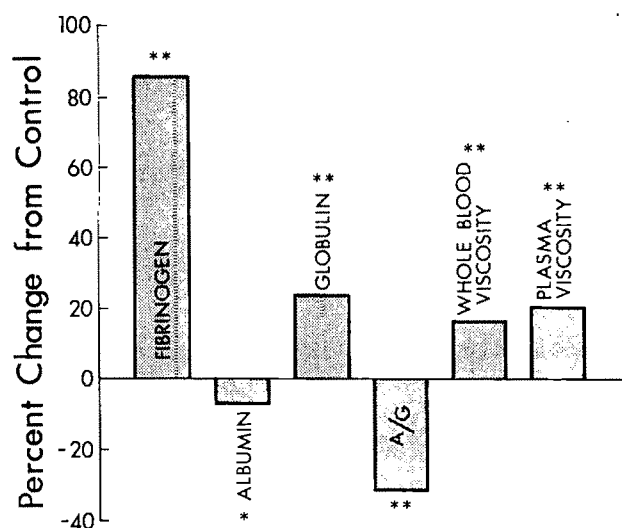


Fig. 3. Percent change from control values for fibrinogen, albumin, globulin, albumin-to-globulin ratio, and whole blood and plasma viscosities. * = $p < 0.05$; ** = $p < 0.01$.

chronic administration of 17β -estradiol resulted in decreased mean arterial pressure, increased heart rate, expansion of the interstitial space as reflected by the body weight, and expansion of the blood volume agree with the general mechanisms for volume expansion proposed previously.^{4, 20, 21} Although the circulating doses of estrogen in the present study were greater than the physiologic, this should not detract from the value of the study because we were attempting to demonstrate an effect in a time length shorter than the normal duration of pregnancy.

Plasma proteins and blood viscosity. The second aim of the present study was to test the hypothesis that plasma protein concentrations and blood viscosity can reflect circulating estrogen concentrations. Human pregnancy is associated with marked changes in plasma protein concentrations. For instance, fibrinogen and globulin concentrations increase about 50% and 13%, respectively, while albumin decreases about 30%, so that the albumin-to-globulin ratio decreases about 36%.^{13, 26} Also, in women using oral contraceptives the increase of fibrinogen and globulin concentrations has been alleged to result from the elevated circulating estrogen concentrations.^{27, 28} In this study 17β -estradiol infusion resulted in plasma protein changes similar to those observed during the course of gestation and in women on oral contraceptives (Fig. 3 and Table II).

Because both fibrinogen and globulin are known to increase both plasma and whole blood viscosity,^{29, 30} the substantial viscosity increases observed in the present study are not surprising. Additionally, the moderate decline in albumin would enhance viscosity.^{23, 30} These results vary somewhat from the findings reported in pregnancy. During pregnancy the changes in whole blood viscosity are more complex than those in plasma, because the hematocrit decreases as pregnancy pro-

ceeds. These hematocrit changes are superimposed on any baseline changes in plasma viscosity. Because a declining hematocrit decreases whole blood viscosity, this effect counteracts an increase resulting from plasma protein changes. Such an interaction between a rising plasma viscosity and a falling hematocrit would account for the decline in whole blood viscosity that most investigators have noted as term approaches.^{12, 31-34}

Not all authors are, however, in agreement on whether whole blood viscosity declines during pregnancy.³⁵ At least part of this disagreement can be attributed to differences in the shear rate at which the viscosity determinations were carried out. During measurements made at low shear rates the red cells interact in such a fashion that the apparent whole blood viscosity is increased. Increases in fibrinogen enhance this aggregation and thus increase the measured viscosity of whole blood in at least two ways: The first is by directly enhancing plasma viscosity. The second is by enhancing cell-cell interaction, particularly at low shear rates. Capillary viscometers such as the one employed in this study are inherently high shear devices and as such are quite insensitive to cell-cell interactions. Because of this characteristic they respond to the increase in plasma viscosity that a heightened fibrinogen will cause but are unaffected by any viscosity increase resulting from fibrinogen-enhanced red cell aggregation.

Measurements of plasma viscosity are more straightforward, and the results obtained are essentially independent of the shear rate applied, because cell-cell interactions are excluded. Furthermore, in a series of measurements made over time there are no variations in hematocrit to complicate the interpretation. It is thus surprising that during the course of pregnancy some authors report no change or a slight increase,³⁶ others report a decrease,³⁴ and still others claim to have ob-

served an increase during early pregnancy followed by a decline at term.³¹

From this confusion in the literature it is clear that no experimental model, even a complex one, will succeed in reproducing such discordant results. The present studies do, however, provide strong evidence that estrogen, in isolation, produces a marked rise in fibrinogen and globulin, a moderate decrease in albumin, and an unequivocal enhancement of plasma viscosity. As such, the findings may help to sort out the effects of the hormonal changes that occur in women taking oral contraceptive medication.³⁷ They may also, if due respect is accorded the more complex hormonal interplay surrounding pregnancy, provide some understanding of both the normal physiology and the pathophysiology of this state.³⁸⁻⁴⁰

In conclusion, estrogen administration reproduced several of the hemodynamic changes observed in human pregnancy. A perfect reproduction of these alterations cannot be expected because of differences in species and the overall hormonal milieu.

We propose that during pregnancy increasing estrogen concentrations decrease systemic vascular resistance and mean arterial pressure, with increased glomerular filtration rate. Estrogen may also affect the lymphatic vessels and interstitial space compliance, as evidenced by the fact that most of the fluid retained is in the interstitial space. These changes result in expanded plasma and interstitial fluid volumes. A possible mechanism for these changes would be as follows: estrogen results in increases in renin, angiotensin, and aldosterone with resultant fluid retention. Estrogen administration has been shown to decrease the number of angiotension receptors and to increase the amount of vasodilatory prostaglandins in the uterine vein of castrated rabbits treated with estrogen. A combination of both factors would result in decreased tone in blood and lymphatic vessels, with decreased arterial pressure and increased heart rate. Uteroplacental vascular shunts may also contribute to these changes.

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Pregnancy-induced hypertension: Development of a model in the pregnant sheep

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Selected hemodynamic, renal, and biochemical parameters were assessed in chronically instrumented third-trimester pregnant ewes and in the same ewes after induction of pregnancy toxemia. Ewes with induced pregnancy toxemia developed hypertension, proteinuria, ketonuria, decreased glomerular filtration rate, decreased cardiac output, and decreased left uterine artery blood flow. Histological and transmission electron microscopy revealed the development of renal morphologic changes consistent with those observed in human pregnancy-induced hypertension. These studies have elucidated that pregnancy-induced hypertension can be produced experimentally in the pregnant ewe. Furthermore, the pathophysiologic features of ovine pregnancy toxemia are similar to those of human preeclampsia, and therefore the sheep provides a suitable animal model to study the human condition, which still remains a major complication of pregnancy, jeopardizing both mother and fetus. (*AM J OBSTET GYNECOL* 1986;155:201-7.)

Key words: Pregnancy-induced hypertension, sheep, preeclampsia, renal

Toxemia states of pregnancy are known to occur in various animal species although none to date has been

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found to be a precise model for human preeclampsia. The human condition is a hypertensive disorder peculiar to pregnancy and has been associated with proteinuria,¹ a decrease in blood volume,² thrombocytopenia,³ uteroplacental ischemia,⁴ prostaglandin deficiency,^{5,6} increased thromboxane A₂ production,⁷ increased activity of the sympathetic nervous system⁸ and the renin-angiotensin system,⁹ and renal morphologic changes.¹⁰

Several investigators have compared ovine pregnancy toxemia to human pregnancy-induced hypertension. In the ewe, the toxic syndrome of pregnancy demonstrates a close resemblance to human preeclampsia with proteinuria and uteroplacental isch-

emia,¹¹ decreased glomerular filtration rate,¹² and coexistent renal morphologic changes¹³ having been implicated. The difference between ovine pregnancy toxemia and human preeclampsia to date is that hypertension has not been demonstrated in the ewe. Previously two studies have investigated whether hypertension develops during ovine pregnancy toxemia.^{13, 14} One group¹³ found no rise in blood pressure during ovine pregnancy toxemia. However, this study suffers from old techniques of measuring blood pressure, and apparently measurements of blood pressure were recorded after the ewes demonstrated neurological involvement, paralysis, and shock. Morris et al.¹⁴ measured blood pressure in gravid ewes before and then after a 5-day fast. However, they did not measure pressures continuously during the fasting period.

Certainly development of a convincing animal model of human pregnancy-induced hypertension, such as the ewe, would be an important step toward understanding the human condition. The ovine model would have a variety of advantages including convenience, availability, and cost. The present experiments were designed to elucidate the pathophysiologic features of ovine pregnancy toxemia in the hope of providing a precise animal model for study of human pregnancy-induced hypertension.

Material and methods

Five third-trimester pregnant ewes, with gestations of 130 to 135 days (term 146 to 150 days), were obtained from Whitehouse Farms, Luray, Virginia, and conditioned to metabolic crates. They received identical diets consisting of hay, concentrate, and water as desired and were housed in an identical environment during the experimental period. The ewes were instrumented, and after recovery from the surgical procedures selected hemodynamic, renal, and biochemical parameters were assessed in the ewes during feeding and in the same ewes after induction of pregnancy toxemia by withdrawal of feed but not water. These parameters were also examined in three nonpregnant ewes during feeding and after a 72-hour fast.

Hemodynamic studies. At 137 to 142 days of pregnancy the ewes were instrumented with the use of aseptic techniques and local anesthesia. A polyethylene catheter was placed in the aorta via the left carotid artery through a small incision in the neck. A Gould Statham physiologic pressure transducer and a Grass Model 7D polygraph recorder were used to measure systolic, mean, and diastolic blood pressures. The heart rate was calculated from the arterial pulse tracings. The aortic catheter was exteriorized and affixed to the neck.

A Swan-Ganz thermal dilution catheter was placed percutaneously into the pulmonary artery via the ex-

ternal jugular vein to measure cardiac output by the thermal dilution method with an Elecath cardiac output computer. One port of the Swan-Ganz catheter was used for the purpose of blood withdrawal from the jugular vein. Total peripheral resistance was calculated by dividing mean arterial pressure by cardiac output and then multiplying by 80 so that the final units were in dyne · sec cm⁻⁵.

A standing left paralumbar fossa laparotomy was performed in three of the pregnant ewes under local anesthesia. The left uterine artery was isolated and an electromagnetic flow probe was placed around the artery to determine qualitative changes in uterine blood flow with a Biotronex Laboratory flowmeter. The laparotomy incision was closed, and the blood flow probe was exteriorized through the dorsal aspect of the incision. After surgical preparation the ewes were allowed to recover for 3 to 4 days before recordings were begun.

Glomerular filtration rate determination. An indwelling sterile urethral catheter was inserted and glomerular filtration rate was measured by an exogenous creatinine clearance determination. Briefly, creatinine (100 mg/ml) was injected subcutaneously and then 40 minutes later urine was collected for 20 minutes via the urethral catheter. Blood was collected at the beginning and end of the 20-minute collection period and along with the urine was analyzed for creatinine content. Creatinine clearance (C_c) was calculated by the following formula:

$$C_c = \frac{U \times V}{P}$$

where U = urine concentration of creatinine (milligrams per milliliter), V = urine flow (milliliters per minute), and P = plasma creatinine concentration (milligrams per milliliter). If it is assumed that there was no reabsorption or secretion of creatinine by the renal tubules, creatinine clearance equaled glomerular filtration rate.

Kidney biopsy and histopathologic studies. Biopsy specimens of the left kidney of three of the pregnant ewes were obtained with a true-cut biopsy instrument through a small incision in the left paralumbar fossa with the animals under local anesthesia at the time the ewes exhibited clinical signs of pregnancy toxemia. Biopsy specimens obtained at the time of instrumentation before feed withdrawal and induction of pregnancy toxemia served as controls. Kidney biopsy specimens were fixed in modified McDowell-Trump's fixative (4% formaldehyde and 1% glutaraldehyde in 280 mOsm/L of Tyrode's buffer, pH 7.4). The biopsy specimens were postfixfixed overnight in 1% osmium tetroxide. The specimens were washed in Tyrode's buffer, dehydrated in a graded ethanol series, and embedded in epoxy resin.

Table I. Selected maternal hemodynamic and renal values at baseline and during induced hypertension (mean $\pm$ SE)

Parameter	Baseline	Hypertension
Cardiac output (L/min)	8.82 $\pm$ 0.32	8.06 $\pm$ 0.39
Peripheral resistance (dyne $\cdot$ sec $\cdot$ cm ⁻⁵)	910 $\pm$ 47	1206 $\pm$ 52
Middle uterine artery flow (ml/min)	375 $\pm$ 75	275 $\pm$ 61
Glomerular filtration rate (ml/min)	141 $\pm$ 8	110 $\pm$ 16

Ultrathin (60 to 80 nm) sections stained with lead citrate and uranyl acetate were examined by transmission electron microscopy. The glomeruli, tubules, interstitium, and blood vessels were examined in detail. In addition, each biopsy specimen was stained with methylene blue-azure II-basic fuchsin for light microscopic examination.

Urinalysis. Urine was collected by catheterizing the bladder and the levels of protein, glucose, ketones, and pH were determined by Labstix reagent strips (Ames Company). Urinalysis was performed at 8-hour intervals throughout the experimental period. Urine samples collected before the induction of pregnancy toxemia were used as controls.

Serum biochemical studies. Blood samples were drawn from the jugular vein every 8 hours throughout the experimental period for the determination of biochemical profiles. These profiles measured serum levels of albumin, total protein, creatinine, blood urea nitrogen, glucose, and aspartate aminotransferase. The serum values for the following electrolytes were determined: calcium, magnesium, phosphorus, bicarbonate, chloride, potassium, and sodium. Serum samples drawn preceding the induction of pregnancy toxemia were used as control values.

Statistical analysis. Each ewe (pregnant and nonpregnant) served as its own control and statistical analysis was performed by paired *t* test comparisons. Probability of ≤ 0.05 was considered to be significant. All results are reported as mean $\pm$ SE.

Results

The three nonpregnant ewes demonstrated no significant changes in the measured hemodynamic, renal, and biochemical parameters during feeding as compared with values after a 72-hour fast.

Hemodynamic responses. Mean aortic pressures were measured by indwelling catheters in third-trimester pregnant ewes during feeding and in the same ewes after induction of pregnancy toxemia by feed withdrawal and are presented in Fig. 1. Mean arterial pressure rose significantly in all the ewes from 95 $\pm$ 7.7

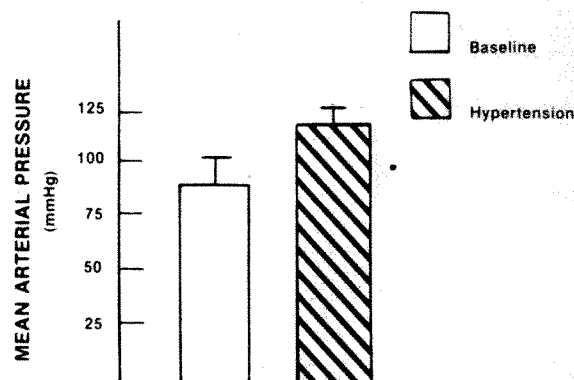


Fig. 1. Mean arterial pressure (mean $\pm$ SE) is presented. During induced hypertension a significant increase ($p < 0.05$) in pressure occurred.

mm Hg during feeding to 117 $\pm$ 1.1 mm Hg after induction of pregnancy toxemia ($p < 0.05$). During pregnancy-induced hypertension the mean arterial pressures had increased by 19% as compared with baseline values. In the three nonpregnant ewes, the arterial pressures remained relatively stable (95 mm Hg) during the entire experimental period.

There was no significant change in the heart rate of the pregnant or nonpregnant ewes during the entire experimental period when compared with baseline values. Cardiac output decreased slightly in the pregnant ewes from 8.82 $\pm$ 0.64 L/min during feeding to 8.06 $\pm$ 0.78 L/min after induction of preeclampsia ($p < 0.03$) (Table I). Total peripheral resistance in the pregnant ewes, which was calculated by dividing mean arterial pressure by cardiac output and then multiplying by 80, also increased significantly from 910 $\pm$ 94 dyne $\cdot$ sec cm⁻⁵ during feeding to 1206 $\pm$ 104 dyne $\cdot$ sec cm⁻⁵ after induction of pregnancy toxemia ($p < 0.01$) (Table I). No significant difference in either cardiac output or total peripheral resistance was demonstrated in the nonpregnant ewes.

Uteroplacental blood flows were measured by an electromagnetic flow probe placed around the left uterine artery in three of the pregnant ewes during feeding and in the same ewes after induction of pregnancy toxemia by feed withdrawal. Left uterine artery blood flows decreased from 375 $\pm$ 61 ml/min during feeding to 275 $\pm$ 50 ml/min after induction of preeclampsia ($p < 0.05$) (Table I). During pregnancy-induced hypertension blood flow through the left uterine artery had decreased 27% as compared with baseline values. Uterine artery blood flows were not measured in the three nonpregnant ewes.

Glomerular filtration rates. Glomerular filtration rates in the pregnant ewes, which were measured by exogenous creatinine clearance determination, were

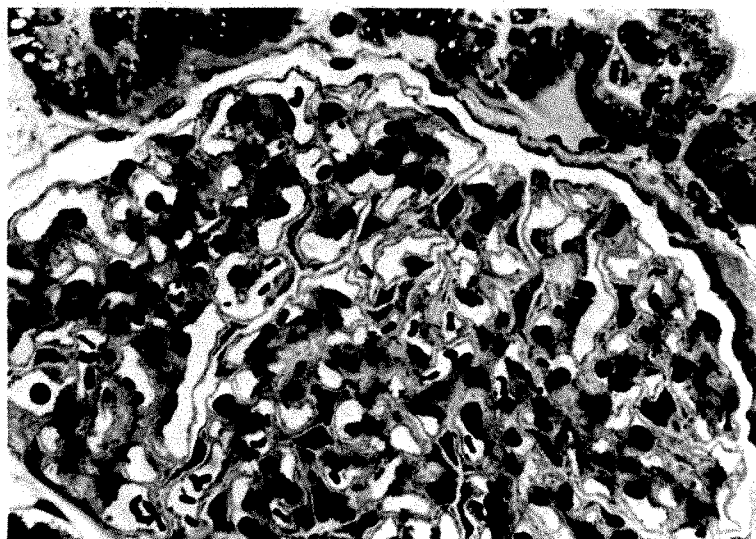


Fig. 2. Biopsy specimen of a kidney from a gravid ewe with induced hypertension. A light photomicrograph showing apparent glomerular swelling encroaching on Bowman's space. The capillary tufts are compressed, and mesangial cells appear to be larger than normal. (Original magnification $\times 160$.)

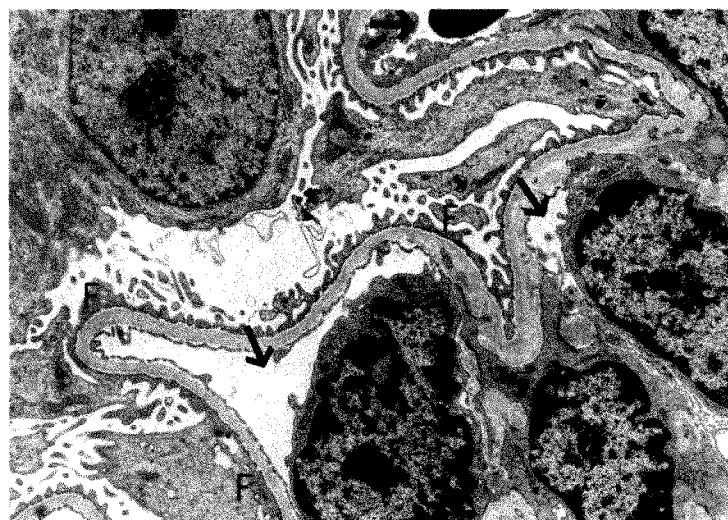


Fig. 3. Biopsy specimen of a kidney from a gravid ewe with induced hypertension. A transmission electron micrograph showing compression of glomerular capillary lumina (arrows). Fusion (F) of mesangial cell podocytes is noted along the basal lamina. (Original magnification $\times 8,000$.)

decreased 22%, from 140 ± 8 ml/min during feeding to 110 ± 16 ml/min during pregnancy-induced hypertension (Table I). The observed decreases in glomerular filtration rates during pregnancy toxemia, however, were not statistically significant.

Histopathologic features of the kidney. The kidney biopsy specimens were examined by light and transmission electron microscopy. Kidney biopsy specimens obtained before feed withdrawal and the induction of pregnancy toxemia served as controls and were unremarkable. Histologically, glomeruli from kidneys of ewes after induction of pregnancy toxemia were swollen,

and the capillary tufts were compressed by swollen mesangial cells (Fig. 2). Transmission electron microscopy revealed fusion of mesangial cell podocytes along the basal lamina of the glomerular capillaries (Fig. 3). Electron-dense deposits observed in the mesangial cell podocytes probably represent protein (Fig. 4). Proximal tubular cells had few microvilli and few micropinocytotic vesicles. Within the proximal tubule cells the ribosomes were aggregated and abundant matrix granules were present within the mitochondria (Fig. 5).

Urinalysis. Urine samples, which were collected before the induction of pregnancy toxemia, were used as

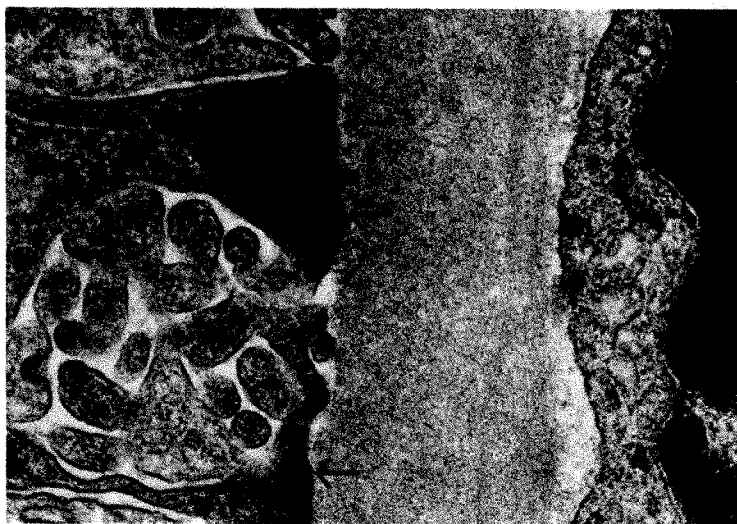


Fig. 4. Biopsy specimen of a kidney from a gravid ewe with induced hypertension. This transmission electron micrograph shows electron-dense deposits (*arrows*) in mesangial cell podocytes, probably representing protein. (Original magnification $\times 29,000$.)

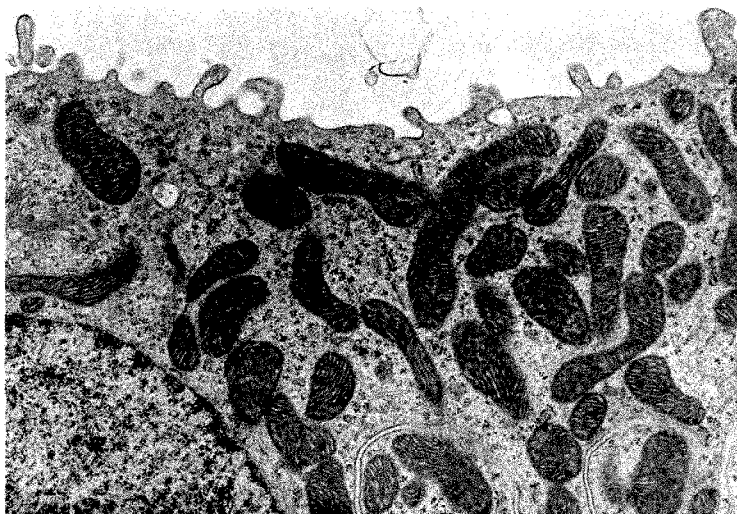


Fig. 5. Biopsy specimen of a kidney from a gravid ewe with induced hypertension. This transmission electron micrograph of an abnormal proximal tubular cell shows decreased numbers of microvilli, aggregated ribosomes, and many matrix granules within mitochondria. (Original magnification $\times 20,000$.)

controls and were either negative or contained a trace amount of ketones. Urinalysis in these control ewes was negative for glucose, and mean pH ranged from 6.0 to 8.5. The animals demonstrated a proteinuria that averaged approximately 1.5+ (Fig. 6). After the induction of pregnancy toxemia, urinalysis showed large quantities of ketones, negative glucose, and a tendency for the pH to decrease to <6.0 . Proteinuria was present in all the ewes and averaged approximately 3.5+ (Fig. 6), which was significantly elevated compared with that of control ewes ($p < 0.03$).

Serum biochemical studies. Serum levels of albumin,

total protein, creatinine, blood urea nitrogen, aspartate aminotransferase, and electrolytes did not change significantly during the course of the experiment. Serum glucose, however, significantly decreased after induction of pregnancy toxemia (fed, 57.6 ± 7.32 mg/dl; fasted, 27 ± 3.85 mg/dl; $p < 0.03$).

Comment

The information derived from our present study clearly demonstrates that pregnancy-induced hypertension can be produced in third-trimester pregnant ewes by withholding feed for 72 hours. Kaufman and

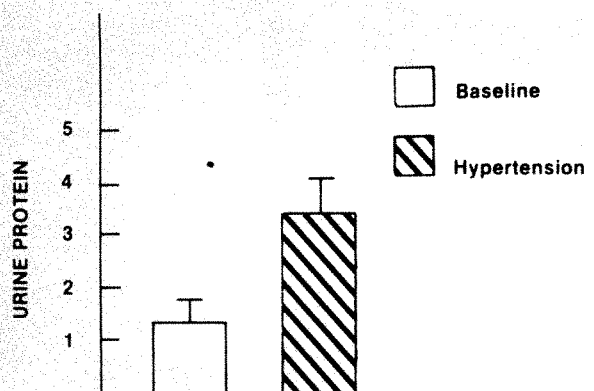


Fig. 6. Relative urine protein content is presented. During induced hypertension urine protein was increased ($p < 0.03$).

Bergman¹² demonstrated in ewes with twin pregnancies that feed withdrawal for 72 hours was sufficient to produce varying degrees of pregnancy toxemia. The finding that pregnancy-induced hypertension does occur in the ewe is an important step in developing the ewe as a convincing animal model to study human preeclampsia. The development of hypertension, the decrease in cardiac output, the increase in total peripheral resistance, and the decrease in middle uterine artery blood flow after induction of pregnancy toxemia in the ewe are hemodynamic responses that are consistent with human pregnancy-induced hypertension.

Uteroplacental blood flow has been demonstrated to decrease in human preeclampsia.¹⁵ Uteroplacental ischemia is a major reason for the high incidences of fetal loss, intrauterine growth retardation, and small-for-dates infants observed in women with pregnancy-induced hypertension. Fowden and Silver¹¹ measured uterine blood flows in four ewes carrying single fetuses before and at 24 and 48 hours after food withdrawal at 134 and 137 days of gestation. These investigators demonstrated no consistent changes in uterine blood flow during the 48-hour period of food withdrawal. These ewes did not demonstrate clinical signs of pregnancy toxemia. In our study, however, left uterine artery blood flow was significantly decreased after 72 hours of food withdrawal and the induction of pregnancy toxemia.

In the present study, the glomerular filtration rate tended to decrease with the induction of pregnancy toxemia although the decrease was not significant. Kaufman and Bergman¹² demonstrated that glomerular filtration rate was significantly diminished to about 25% in ewes with twin pregnancies that were fasted. Glomerular filtration rate declined as the severity of the toxemia increased.

Histopathologic evaluation of kidney biopsy specimens from fed third-trimester ewes showed no pathologic lesions. In contrast, after induction of pregnancy

toxemia in these same ewes, there were renal changes that mainly affected the mesangial cells, resulting in compression of the glomerular capillary lumina. These findings are interesting in view of the evidence that a population of mesangial cells functions as smooth muscle cells to regulate glomerular filtration in response to local stimuli.¹⁶ These authors' results further suggest that this population of mesangial cells predominates in pregnancy-induced hypertension and also demonstrates characteristic interrelationships with constricted capillaries and with fusion of endothelium overlying mesangial regions. These findings support the theory that alterations in the contractile responsiveness of "myogenic" mesangial cells play a pathogenic role in initiating glomerular and vascular dysfunction in human preeclampsia. The renal morphologic lesions of ovine pregnancy toxemia and human preeclampsia have been termed glomerular endotheliosis.^{10, 13} The histologic fine structural appearance of the kidney seen in ovine pregnancy toxemia¹³ is very similar, if not identical, to those seen in human pregnancy-induced hypertension.

Abnormal proteinuria almost always accompanies human preeclampsia¹ and ovine pregnancy toxemia.¹³ Proteinuria may be minimal, moderate, or severe. Proteinuria in preeclampsia has been attributed to vasospasm or to the glomerular lesion that accompanies the disease. The latter is supported by the fact that the magnitude of proteinuria correlates with the severity of the morphologic lesion.

In conclusion, our results demonstrate that pregnancy toxemia of the ewe is a hypertensive disorder peculiar to pregnancy and is associated with uteroplacental ischemia, proteinuria, and renal morphologic changes that are similar to human pregnancy-induced hypertension. Our clinical experience with ovine pregnancy toxemia has been that ewes carrying twins are very susceptible to pregnancy toxemia in the final 2 weeks of gestation. Their disease is most severe and immediate cesarean section is the only effective treatment. Data from our present sheep study seem to be in accordance with the results obtained from human investigations, making the ewe an excellent animal model to study the human disorder. Elucidation of the pathophysiologic features of ovine pregnancy toxemia has provided a precise animal model for study of human pregnancy-induced hypertension, which still remains a major complication of pregnancy, jeopardizing both mother and fetus.

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The effect of ethanol on the production of lactate, triglycerides, phospholipids, and free fatty acids in the perfused human placenta

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Eighteen fresh term placentas from normal deliveries were perfused to determine the biochemical response of the human placenta in the absence and presence of ethanol. The production rates were generally increased by ethanol administration; lactate, 22%; triglycerides, 49%; sphingomyelin, 362%; lecithin, 67%; lipid phosphorus, 780%; free fatty acids, 897%. Only the changes in lipid phosphorus and free fatty acids were significantly different ($p < 0.05$). Glucose utilization rates were 600 and 551 mg per hour per placenta for eight control and 10 ethanol runs, respectively. The ethanol utilization rate was 442 mg per hour per placenta. Little difference in ultrastructural appearance between control and ethanol groups was observed at the level of ethanol administered. The general behavior of ethanol metabolism in human placenta seems qualitatively similar to that in the liver. (*AM J OBSTET GYNECOL* 1986;155:207-11.)

Key words: Human placenta, ethanol, perfusion, utilization, production

The deleterious effects on the fetus that characterize the fetal alcohol syndrome and the use of ethanol to prevent premature labor have served to stimulate studies of the effect of ethanol on the fetus during its de-

velopment.^{1,2} Ethanol is normally metabolized by the liver with no more than 20% of ethanol metabolism occurring in extrahepatic sites.² The rate-limiting step in the ethanol metabolism involves alcohol dehydrogenase and its cofactor nicotinamide adenine dinucleotide. If ethanol is metabolized similarly by the placenta, the enhanced reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide ratio induced by ethanol may alter the spectrum of biochemical products that result from its metabolism. These products in turn may play an extremely important part in the regulation of the biochemical processes in the fetus. However, only a limited number of reports have been found

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Table I. Utilization and production rates of human placenta in absence of ethanol

Placenta no.	Weight (gm)	Glucose	Lactate	Triglycerides	Lipid phosphorus	Sphingomyelin	Lecithin	Free fatty acids
1	486	654	239	169	—	-2.9	16.4	—
2	734	545	267	130	—	6.1	-1.7	—
3	730	345	307	42	—	-0.4	5.9	—
4	529	636	352	140	0.48	—	—	—
5	503	343	172	265	0.38	—	—	—
6	498	828	372	160	0.21	—	—	5.3
7	492	618	284	180	1.17	8.1	23.3	30.0
8	627	834	606	189	1.94	6.1	15.0	72.0
Mean $\pm$ SE	575 $\pm$ 38	601 $\pm$ 66	325 $\pm$ 46	159 $\pm$ 22	0.84 $\pm$ 0.32	3.4 $\pm$ 2.1	11.8 $\pm$ 4.4	35.8 $\pm$ 19.0

Utilization rate is for glucose only; production rate is given for others. All rates are given as milligrams per hour per placenta, except those for sphingomyelin and lecithin, which are only relative amounts and expressed without dimension.

Table II. Utilization and production rates of human placenta in presence of ethanol

Placenta no.	Weight (gm)	Glucose	Lactate	Tri-glycerides	Lipid phosphorus	Sphingo-myelin	Lecithin	Free fatty acids	Ethanol
1	614	726	340	232	—	7.0	-8.3	—	—
2	521	840	371	309	—	15.2	30.8	—	549
3	730	858	394	223	—	—	—	—	555
4	495	304	404	136	—	—	—	—	798
5	658	292	361	134	—	—	—	—	350
6	448	166	306	179	5.50	5.1	10.9	—	308
7	542	521	338	281	3.65	11.7	8.8	—	255
8	464	624	364	221	13.0	20.4	34.4	—	302
9	465	491	371	142	6.84	8.8	14.2	258	209
10	665	690	714	513	7.62	42.0	46.9	456	470
Mean $\pm$ SE	560 $\pm$ 31	551 $\pm$ 76	396 $\pm$ 36	237 $\pm$ 36	7.32 $\pm$ 1.56	15.7 $\pm$ 4.8	19.7 $\pm$ 7.0	357 $\pm$ 99	422 $\pm$ 63
p Value	0.77	0.64	0.23	0.10	0.0037	0.067	0.41	0.026	—

Utilization rates are for glucose and ethanol; and production rate is given for others. All rates are given as milligrams per hour per placenta, except those for sphingomyelin and lecithin, which are only relative amounts and expressed without dimension. p Values were determined by the Student *t* test for unpaired data with equal population variances.

in the literature concerning the biochemical pattern of the human placenta accompanied by ethanol perfusion. Investigations of the ethanol utilization rate and the lipid production rate have not been reported in the human placenta.

The purpose of this study was to measure these biochemical responses in the absence and presence of ethanol. Utilization rates for glucose and ethanol and production rates for lactate, triglycerides, phospholipids, lipid phosphorus, and free fatty acids were determined for 18 human placentas at term. Concomitantly, placental ultrastructures were observed to ascertain any structural changes induced by ethanol.

Material and methods

Eighteen fresh term placentas from either cesarean section or spontaneous labor and delivery were perfused with Krebs-Ringer bicarbonate solution within 10 minutes after delivery. The dual perfusion apparatus described elsewhere^{3,4} was used to establish physiologic levels of glucose, pH, PO_2 , and temperature during the first 30 minutes. The maternal and fetal flow rates were

600 and 30 ml/min, respectively. Glucose was dripped continuously into the maternal reservoir at a rate of 600 mg/hr, approximately equal to the rate of glucose utilization by the placenta.⁵ In the ethanol runs, 6.3 gm of absolute ethanol was added to 2 L of maternal perfusate after a 30-minute equilibrium period. During both the control and the ethanol runs, samples were taken from the maternal and fetal reservoirs every 15 minutes. All samples were centrifuged promptly and their supernatants were frozen until analysis the following day.

Glucose, ethanol, lactate, and triglycerides were assayed by standard enzymatic kits from Sigma Chemical Company, St. Louis, Missouri. Phospholipids were determined by one-dimensional thin-layer chromatography.⁵ Silica gel H plates with 5% ammonium sulfate were purchased from Analtech, Newark, New Jersey. L- α -phosphatidyl-DL-glycerol from egg yolk lecithin and synthetic DL- α -lecithin were purchased from Sigma to calibrate the position of phospholipids on the plates. The relative magnitudes of sphingomyelin and lecithin were determined for the lecithin/sphingomyelin ratio.

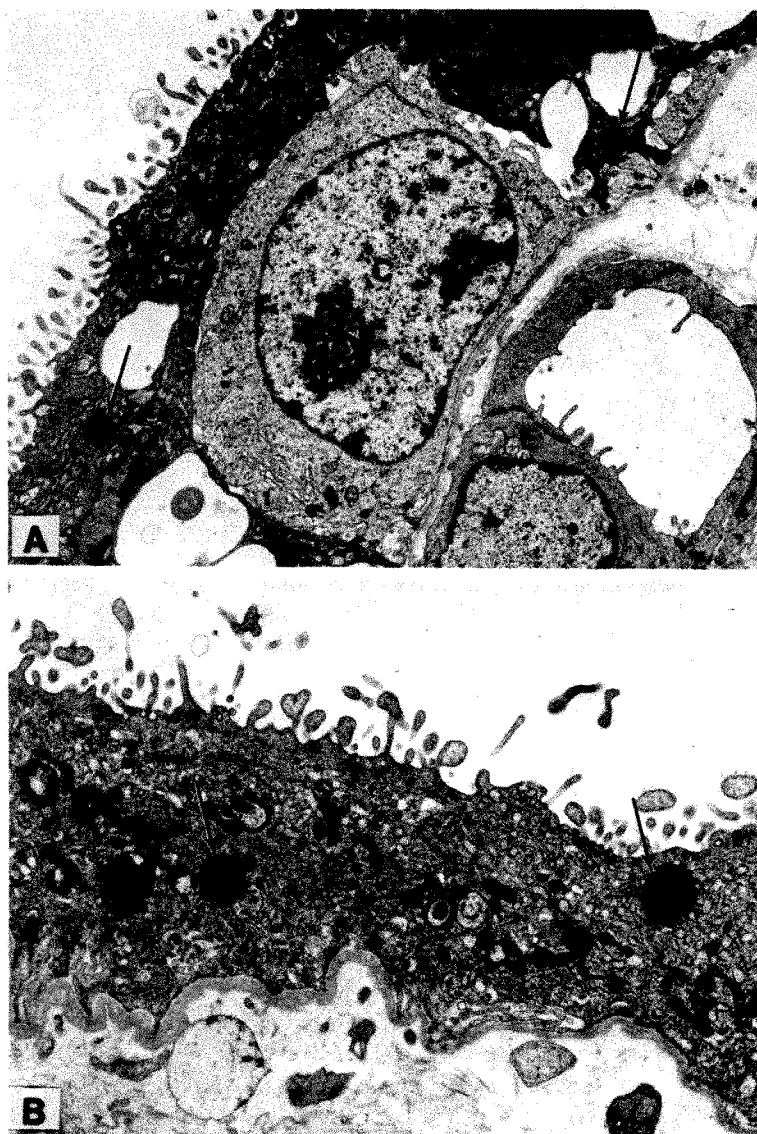


Fig. 1. Ultrastructure of placental tissues for control (A) and ethanol (B) after 270 minutes of perfusion. S, Syncytiotrophoblast with preserved microvilli; C, cytotrophoblast; E, capillary endothelium. Arrow: Lipid droplets (A, Original magnification $\times 10,900$. B, Original magnification $\times 13,600$.)

Lipid phosphorus was measured by the ascorbic acid method.⁵ A standard solution of potassium dihydrogen phosphate, 2 mg/ml, was run simultaneously with samples to be analyzed. Free fatty acids were determined by the method used by Dancis et al.⁷ The placental tissues for electron microscopy were sampled at the end of each perfusion and fixed in cold phosphate-buffered 2.5% glutaraldehyde. These samples were sequentially washed with 0.2 mol/L phosphate buffer, postfixed with 1% osmium tetroxide, embedded in araldite, and stained with uranyl acetate and lead citrate before observation of the placental ultrastructure in a Philips EM300 electron microscope. The statistical significance

was determined by the Student *t* test for unpaired data with equal population variances. Differences between control and ethanol groups were considered significant when $p < 0.05$.

Results

The total quantity used or produced by the placenta was computed by the mass balance for each compound: glucose, ethanol, lactate, triglycerides, sphingomyelin, lecithin, lipid phosphorus, and free fatty acids. Each rate of utilization or production was then determined by its slope of total quantity versus time curve. Eight individual placentas were used as a control group per-

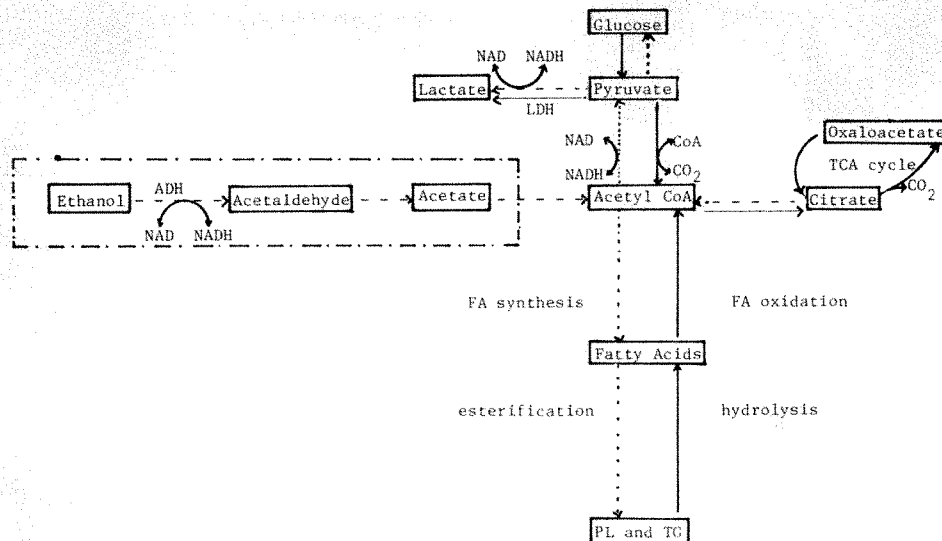


Fig. 2. Metabolic pathways of glucose and ethanol metabolism in the human placenta. *ADH*, Alcohol dehydrogenase; *LDH*, lactate dehydrogenase; *TCA*, tricarboxylic acid; *PL*, phospholipids; *TG*, triglycerides; *NAD*, nicotinamide adenine dinucleotide; *NADH*, reduced nicotinamide adenine dinucleotide. The pathway is enhanced (--->) or impaired (→) by ethanol administration. The metabolic scheme is modified from Zubay and Zubay.¹⁵

fused in the absence of ethanol (Table I). Ten placentas perfused in the presence of ethanol were considered as an ethanol group (Table II). The rates were compared between each group, and they were generally increased by ethanol administration: lactate, 22%; triglycerides, 49%; sphingomyelin, 362%; lecithin, 67%; lipid phosphorus, 780%; and free fatty acids, 897%. Only the changes in lipid phosphorus and free fatty acid were significantly different. The utilization rate of glucose by the placenta was slightly decreased (−8%). The lecithin/sphingomyelin ratios for control and ethanol groups were 3.5 ± 1.3 and 1.7 ± 0.6 , respectively.

Typical results of the placental ultrastructure are shown in Fig. 1. Little structural difference was observed between the groups. In particular, the surface microvilli, the mitochondria, and the endoplasmic reticulum were similar in both control and ethanol groups. However, the appearance of more lipid droplets in the presence of ethanol was consistent with the results given in Table II.

Comment

Glucose. The glucose utilization rate of the control group, 600 mg per hour per placenta, agrees with previous values.³ No literature directly related to the effect of ethanol on glucose utilization rate in the human placenta was available. Towell and Erwin⁸ reported that glucose utilization rate in the isolated perfused mouse brain was decreased 38% at an ethanol level of 400 mg/dl, which was explained by the absence of compensatory mechanisms occurring in vivo. The present study

indicated only an 8% decrease of glucose utilization rate in the presence of ethanol, which was not significantly different ($p = 0.64$) from the value obtained without ethanol.

Ethanol. An ethanol utilization rate of 422 mg per hour per placenta was observed. No previous measurements of human placental ethanol utilization rate were available in the literature. The unoxidized ethanol from the placenta can reach the fetus and remain there for extended periods, because alcohol dehydrogenase activity in the human fetal liver reaches only 20% of adult activity by term.⁹ This may adversely affect the fetal development during the course of pregnancy.

Lactate. The 22% increase in production rate of lactate agrees qualitatively with the previous work in the perfused rat liver treated with ethanol.¹⁰

Triglycerides. A 49% increase in the triglyceride production rate in the presence of ethanol was observed ($p = 0.10$). The presence of ethanol may divert the main source of adenosine triphosphate production for the cell from fatty acid oxidation to ethanol oxidation, thus raising the reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide ratio to favor triglyceride accumulation.

Phospholipids. The syncytium of the human placenta contains many lipids, mainly in the peripheral and basal parts with two thirds of the fat content of the term placenta consisting of lipids, 76.6% of these being phospholipids. This high lipid content and the use of lipid complexes as carriers of amino acids imply their substantial importance in the function of the placenta. Previous works¹¹ suggest that phospholipids and tri-

glycerides are formed from esterification of fatty acids from maternal blood. The determination of the phospholipid profile in the amniotic fluid, especially the lecithin/sphingomyelin ratio, has served as the standard means of predicting fetal lung maturity (lecithin/sphingomyelin ratio ≥ 2).¹² Although the magnitudes of amniotic and placental phospholipids are different, their ratios of pairs of phospholipid component may be about the same because of the possible equilibrium between amniotic fluid and placental tissues. The ratio of placental production rates of lecithin and sphingomyelin equals the ratio of their concentrations if their initial amounts are negligible compared with total quantities produced during perfusion. Our result of a lecithin/sphingomyelin ratio for the control group was 3.5, which was strikingly close to the work of Nelson et al.¹³ of 3.7 or others. The ratio for the ethanol group was 1.7. This result suggests that the lecithin/sphingomyelin ratio ≥ 2 may not be an absolute method of determining the fetal lung maturity for an alcoholic mother or a pregnant woman treated with ethanol to prevent premature labor.

Lipid phosphorus. The production rate of lipid phosphorus was most significantly different ($p = 0.0037$) between the groups. The production rate of lipid phosphorus increased 8.8-fold after ethanol administration, while the sum of sphingomyelin and lecithin increased only 2.3-fold. This suggests that the distribution of lipid phosphorus among placental phospholipids may be changed in the presence of ethanol. The increased production rate of lipid phosphorus also is consistent with the proposed increased reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide ratio due to ethanol oxidation.

Free fatty acids. Although a significant maternal-fetal transfer of free fatty acids occurs, these may be partly taken up by the placenta and esterified into phospholipids and triglycerides before their transfer to the fetus.¹⁴ Thus the placenta may play an important role in controlling the supply of free fatty acids to the developing fetus. The significantly enhanced production rate ($p = 0.026$) of free fatty acids induced by ethanol administration seems to be caused by the shift of fatty acid oxidation to its synthesis.

Ultrastructure. Jung et al.¹⁵ reported that therapeutic levels of ethanol for prevention of premature parturition range from 90 to 160 mg/dl, intoxication at 200 to 300 mg/dl, and coma at 350 to 500 mg/dl. The present finding of basically the same ultrastructural appearance in both control and ethanol groups may be due to the relatively low level of ethanol administered. The average ethanol concentration was 260 mg/dl in the maternal circulation and 90 mg/dl in the fetal circulation during a typical run.

In summary, the present report appears to be the first wherein the biochemical production rates in the human placenta were observed after a bolus addition of ethanol. The major pathways of glucose and ethanol metabolism in this study are summarized in Fig. 2. Our results in the human placenta suggest that the oxidation of ethanol leads to an elevated ratio of reduced nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide, which in turn leads to increased production of acetyl coenzyme A and subsequently to increased production of free fatty acids and lipid phosphorus. The general behavior of ethanol metabolism in the human placenta seems to be much the same as in the liver.

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The effect of aminophylline on uterine smooth muscle contractility and prostaglandin production in the pregnant rat uterus in vitro

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The effect of aminophylline on contractility and prostaglandin production was investigated in a 20-day pregnant rat uterus preparation in vitro. A significant decrease was observed in frequency (24.4% of control, $p < 0.05$) and contractile force (13.8% of control, $p < 0.001$) in rat uterine segments treated with aminophylline. Treated segments produced more prostaglandin E_2 (139.6% of control, $p < 0.001$) and less prostaglandin $F_{2\alpha}$ (73.8% of control, $p < 0.001$) during the 45-minute observation period. No significant difference in thromboxane B_2 or 6-ketoprostaglandin $F_{1\alpha}$ production was noted. This is the first observation of prostaglandin levels in rat uterine segments treated with aminophylline. It could not be concluded from this study whether the observed changes in prostaglandin production are directly related to the relaxant effect of aminophylline. (AM J OBSTET GYNECOL 1986;155:212-5.)

Key words: Aminophylline, prostaglandins, uterine contractility, pregnant rat uterus in vitro

Aminophylline (theophylline ethylenediamine) is a methylxanthine that relaxes uterine smooth muscle.¹⁻³ Putative mechanisms of action include inhibition of the enzyme phosphodiesterase leading to accumulation of cyclic adenosine monophosphate (cAMP), a known mediator of smooth muscle relaxation, or antagonism of inhibitory adenosine receptors which when stimulated lower cellular cAMP levels.^{4,5}

It has been demonstrated that prostaglandin production is directly related to spontaneous contractile activity in the pregnant rat uterus in vitro.⁶ Ritodrine, an established uterine smooth muscle relaxant, is associated with decreased prostaglandin synthesis in the pregnant rat uterus.⁷ In view of this effect of ritodrine, alternative mechanisms of action to explain uterine smooth muscle response to aminophylline administration were sought. Specifically, pregnant rat uterine contractility and prostaglandin production in vitro following administration of aminophylline were investigated.

Methods

Eight Sprague-Dawley rats with timed pregnancies were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. The rats were sac-

rificed on pregnancy day 20 (day 1 = day after cohabitation). The uterine horn containing the most implantations was removed and slit longitudinally, and fetal, placental, and membranous tissues were discarded. Two full-thickness uterine segments from the same uterine horn, measuring approximately 1×2 cm, were suspended in 25 ml of Krebs bicarbonate buffer, pH 7, at 37° C and bubbled with 95% oxygen and 5% carbon dioxide. One segment served as control, and the other was exposed to aminophylline. The segments were preincubated in the buffer for 10 minutes, after which the buffer was drained and the bath chambers refilled with buffer. A 2 gm tension was then applied to each segment, and 1 mmol/L (0.45 ml) of aminophylline (Lyphomed Inc., Melrose Park, Illinois) was added to the bath of one segment. Via strain gauge transducers attached to the uterine segments, frequency of contractions and integrated contractile force measured in gram-minutes (a measurement equivalent to the area under the contraction curve) were measured and recorded on a Gould polygraph for 45 minutes. In addition, 2 ml of buffer was removed from each bath at the following time intervals: 0 minutes (before aminophylline administration), and then at 5 minutes, 10 minutes, 15 minutes, 30 minutes, and 45 minutes after drug administration. The removed buffer was frozen until assay for the prostaglandins E_2 , $F_{2\alpha}$, thromboxane B_2 , and 6-ketoprostaglandin $F_{1\alpha}$ by radioimmunoassay techniques described in detail previously.^{6,8} After removal of buffer at these intervals, 2 ml of fresh buffer was added to the baths (Krebs buffer to control, Krebs buffer and aminophylline at 1 mmol/L concen-

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tration to treatment) to maintain the bath volumes at 25 ml. Prostaglandin measurements were corrected for this dilution as previously described.⁹ Statistical significance of the data was determined by analyses of variance and two-tailed paired *t* tests.

Results

Contractility. In seven of the eight uterine segments, aminophylline administration to the bath produced an immediate reduction in the frequency of contractions and integrated contractile force. During the 45-minute observation period the mean total number of contractions was reduced in the aminophylline-treated group to $24.4\% \pm 16.3\%$ of the control group ($p < 0.05$). The mean total integrated contractile force was decreased in the segments treated with aminophylline to $13.8\% \pm 9.2\%$ of the control group ($p < 0.001$) (Fig. 1).

Prostaglandins. The concentration of prostaglandin E_2 in the media was greater following aminophylline administration ($139.6\% \pm 18\%$ of control, $p < 0.001$), while prostaglandin $F_{2\alpha}$ concentration was less ($73.8\% \pm 7.8\%$ of control, $p < 0.001$) over the 45-minute experimental period. There was no significant difference in thromboxane B_2 or 6-ketoprostaglandin $F_{1\alpha}$ concentrations between the treated and untreated segments (Fig. 2).

Since concentration changes were significant for prostaglandin E_2 and prostaglandin $F_{2\alpha}$, we made detailed analyses of production (that is, the difference between two sequential concentrations as a function of time). In these analyses we found that the significant changes in production of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ occurred during the first 5 minutes of incubation. Prostaglandin E_2 production increased by $62.5\% \pm 19\%$ ($p < 0.03$) and prostaglandin $F_{2\alpha}$ production decreased by $26.7\% \pm 9\%$ ($p < 0.01$) (Fig. 3).

Comment

There is ample evidence that the theophylline methylxanthines relax pregnant and nonpregnant uterine smooth muscle.¹⁻³ Aminophylline has been used clinically for tocolysis by Liu and Blackwell,¹⁰ who reported favorable results when it was compared to the β -adrenergic agonist salbutamol.

There is debate as to the mechanism of action of methylxanthines in relaxing smooth muscle. Though methylxanthines are known inhibitors of the enzyme phosphodiesterase, they may not exert this effect at therapeutic drug levels in vivo. Mitznegg et al.,³ studying the pregnant and nonpregnant rat uterus in vitro, showed that a 1 mmol/L dose of theophylline relaxed the pregnant uterus by 36% without any demonstrable change in cAMP concentration, whereas higher doses induced relaxation and a dose-dependent increase in cAMP concentration. Polacek et al.¹¹ also failed to dem-

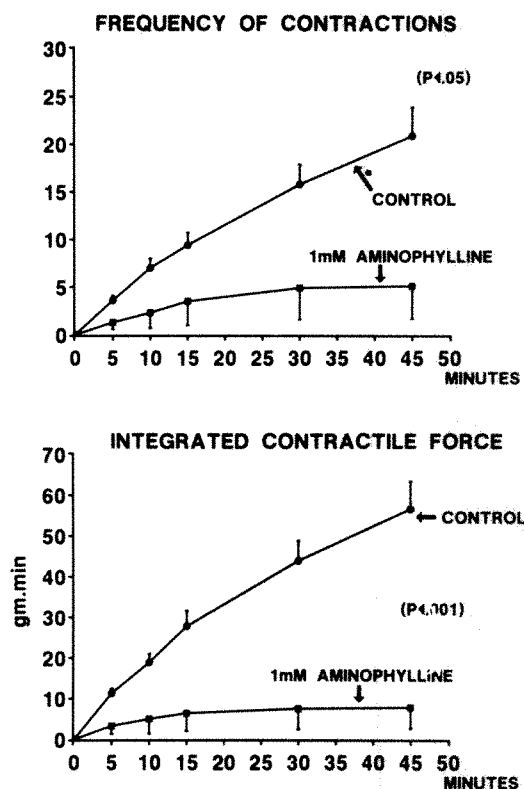


Fig. 1. Mean frequency and integrated contractile force $\pm$ SEM of eight paired uterine segments (p values refer to total values obtained after the 45-minute observation period).

onstrate a change in cAMP concentration associated with theophylline-induced relaxation in uteri of non-pregnant rats. Studying human lung tissue, Bergstrand¹² concluded that at therapeutic concentrations theophylline is a weak inhibitor of phosphodiesterase in vivo.

It has also been demonstrated that theophylline antagonizes adenosine receptors and, in adipocytes, may antagonize the inhibitory adenosine receptor that promotes inhibition of adenylate cyclase and lowered cAMP levels.⁵ Application of this model to uterine smooth muscle requires further investigation.

In previous studies, Dubin et al.⁶ demonstrated that spontaneous contractions of the pregnant rat uterus in vitro are directly related to prostaglandin production. It has also been shown that ritodrine-induced relaxation of pregnant rat uteri is associated with a significant decrease in prostaglandin production (prostaglandin E_2 , prostaglandin $F_{2\alpha}$, thromboxane B_2).⁷ Since dibutyl cAMP relaxed the pregnant rat uterus but increased prostaglandin production (prostaglandin E_2 , thromboxane B_2 , 6-ketoprostaglandin $F_{1\alpha}$), the ritodrine effect on prostaglandin production was thought not to be mediated through adenylate cyclase and cAMP.⁷

Warrick et al.¹³ recently observed significant eleva-

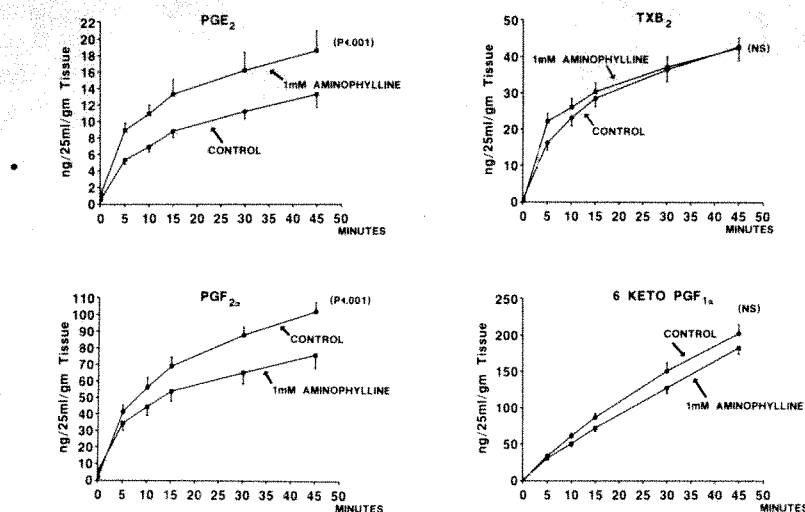


Fig. 2. Mean $\pm$ SEM concentrations of prostaglandins in organ baths of control and aminophylline-treated segments at designated time intervals (p values refer to concentrations over the 45-minute observation period).

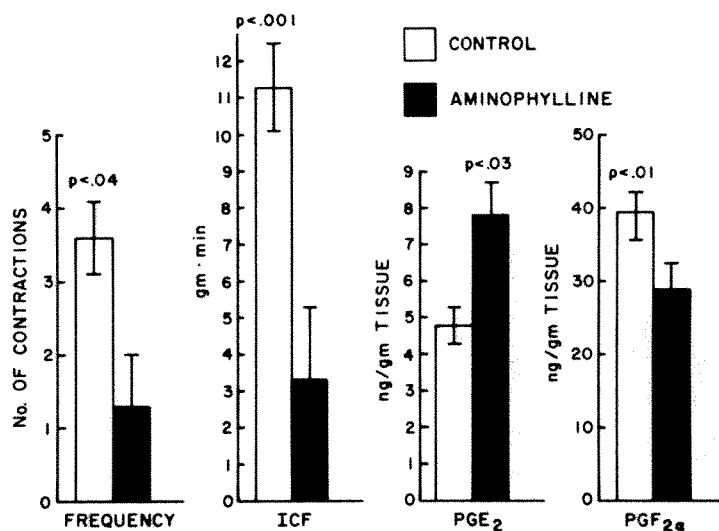


Fig. 3. Mean $\pm$ SEM for frequency, integrated contractile force (ICF), and prostaglandin E₂ and prostaglandin F_{2α} production during the first 5 minutes.

tions of prostaglandins E and F from dispersed human decidual cells incubated with the methylxanthine 3-isobutyl-1-methylxanthine. Similar results were obtained with incubation with dibutyryl cAMP.¹³ The present study differs in that prostaglandin F_{2α} concentration decreased with administration of a methylxanthine. This may be secondary to interspecies variation or distinct effects on prostaglandin production by methylxanthines on endometrium and myometrium.

Aminophylline does not appear to alter prostaglandin levels by affecting cyclooxygenase in view of the observed increase in prostaglandin E₂ levels. A possible site of action might be inhibition of prostaglandin F_{2α}

synthetase with resultant diversion of substrate to the prostaglandin E₂ pathway, or inhibition of 9-ketoreductase which catalyzes the conversion of prostaglandin E₂ to prostaglandin F_{2α}.

The present study explored the possibility that aminophylline-induced inhibition of spontaneous contractions in the pregnant rat uterus in vitro may be mediated via changes in prostaglandin production. Using a 1 mmol/L aminophylline preparation, we observed decreased prostaglandin F_{2α} production but increased prostaglandin E₂ production, both occurring concurrently with relaxation. Since prostaglandin E₂ and F_{2α} are both uterotonic,⁷ it will require further in-

vestigation to determine whether these modest but disparate changes are directly responsible for aminophylline's relaxant effect.

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Amniotic fluid in baboon pregnancies with normal versus growth-retarded fetuses

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Amniotic fluid samples from 12 pregnant baboons at 173 to 176 days of gestation were studied. Five fetuses were growth retarded and seven were normally developed. Mean amniotic fluid volumes and composition (osmolality, protein concentration, delta optical density at 450 nm, and amniotic fluid/maternal plasma creatinine ratio) were similar in the two groups. Growth-retarded fetuses had amniotic fluids with a lower mean ($\pm$ SE) pH than their normally developed peers (7.50 ± 0.083 versus 7.85 ± 0.084 , $p = 0.023$). Intrauterine growth-retarded fetuses appeared to ingest amniotic fluid at a slower mean rate than normally developed fetuses (609 ± 50.9 versus 769 ± 48.6 ml/day, $p = 0.05$), but the difference disappeared when the estimates were corrected for fetal weight. Whether these data may be extrapolated to human pregnancies is speculative but appears likely in view of the similarities between amniotic fluid volume and composition in normal baboon and human pregnancies. (AM J OBSTET GYNECOL 1986;155:216-9.)

Key words: Baboon, intrauterine growth retardation, amniotic fluid, pregnancy

Many perinatal studies may appropriately be performed in an animal model provided that the animal is sufficiently similar to humans in the area to be studied. The considerable similarities in the anatomy, embryology, and physiology of the fetoplacental-uterine units in baboons and humans suggest that the baboon may be an ideal animal in which to investigate problems of human reproduction.¹⁻⁹

The syndrome of fetal growth retardation has been recognized for many years. By the most widely accepted definition, growth retardation affects 10% of fetuses to some degree. Yet information about the intrauterine environment of these babies remains very scarce. The major problem lies in the difficulty of predicting the occurrence of intrauterine growth retardation. In an unselected human population, one would have to study prospectively 100 pregnancies in order to gather data on 10 growth-retarded fetuses. Quantitative information on amniotic fluid volume and composition in growth-retarded versus normally developed fetuses is almost nonexistent. We and others have demonstrated that baboons and humans are very similar in respect to their amniotic fluid environment.^{1, 2, 5} In the course of

experiments with baboons we encountered a number of females who in retrospect were found to bear growth-retarded fetuses. Data on amniotic fluid volume and composition were obtained and compared to similar data from normal pregnancies.

Material and methods

Amniotic fluid from 12 pregnant baboons (*Papio cynocephalus*) was studied at 173 to 176 days of gestation (term: 180 days). Before pregnancy each female was observed daily for extent of erythematous perineal swelling. Mating was allowed during the time of ovulation, and conception time was estimated as 3 ± 2 days before the first day of deturgescence¹⁰ (also Kuehl, unpublished data). On the day of the study each female was sedated with 10 mg/kg of ketamine injected intramuscularly. Positions of the fetus and placenta were located by real-time ultrasonography with a Toshiba SAL 20-A scanner equipped with a 3.5 MHz linear-array transducer. Percutaneous amniocentesis was performed with a 5 cm long, 20-gauge cannula. After removal of a 5 ml sample of amniotic fluid, a sterile solution of 10 gm/dl of inulin was injected, followed by 2 ml of 0.9 gm/dl of sodium chloride as flush. The exact amount of inulin injected was calculated from the measured concentration in the injected solution, the weight of solution administered, and the specific gravity of the injected solution. An average of 1249 mg (range: 1120 to 1562 mg) of inulin were injected. Amniocenteses were repeated under brief ketamine sedation 4, 8, and 24 hours after injection, and 4 ml of amniotic fluid were removed each time. At the time of the first

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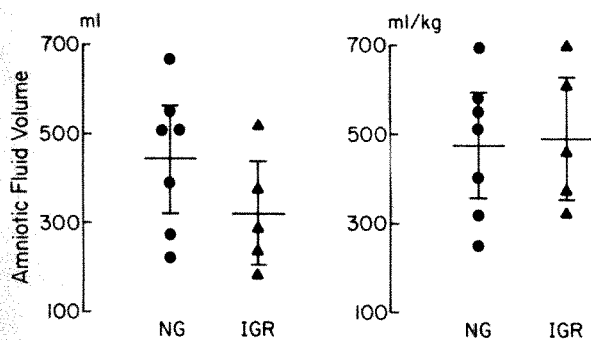


Fig. 1. Effect of intrauterine growth on estimated amniotic fluid volume at term. Mean $\pm$ SE are superimposed on a plot of individual values. (NG, Normal growth; IGR, intrauterine growth retardation.)

amniocentesis a venous blood sample was obtained from the dam by femoral venipuncture.

All blood and amniotic fluid samples were centrifuged for 1 minute at 13,500 rpm in a microcentrifuge. The supernatants were shielded from light and frozen at -15°C until analysis. All chemical analyses were performed in triplicate as previously described.^{1,2} The zero-time concentration of inulin was extrapolated from the 4, 8, and 24 postinjection concentrations with use of the least-squares method for estimating the best fit of these points to a straight line on semilogarithmic plot of concentration versus time.² Amniotic fluid volume was estimated with use of the formula

$$\text{AVF (ml)} = \frac{Q}{C_0}$$

where Q was the amount of inulin injected and C_0 was the zero-time concentration of inulin in amniotic fluid. Fetal swallowing rate of amniotic fluid was calculated from the formula

$$\text{FSR (ml/day)} = \text{AFV} \times \text{slope} \times 24$$

where slope was the slope of the regression line of the natural logarithm of concentration versus time (in milliliters per hour).²

All but two baboons were allowed to deliver spontaneously and did so within 3 days of the study; two baboons were delivered electively by cesarean section. All babies were weighed to the nearest gram. Seven babies were normally developed for gestational age, with mean ($\pm$ SE) birth weight of 927 ± 17.7 gm (range: 854 to 979 gm). Five were intrauterine growth retarded, with birth weights averaging 641 ± 27.1 gm (range: 586 to 745 gm), that is, with birth weights less than two standard deviations below the mean for gestational age.¹¹ The origin of growth retardation was unknown in three cases; in the other two it may have resulted from maternal stress probably induced by weekly anesthesia for various studies from the twenty-

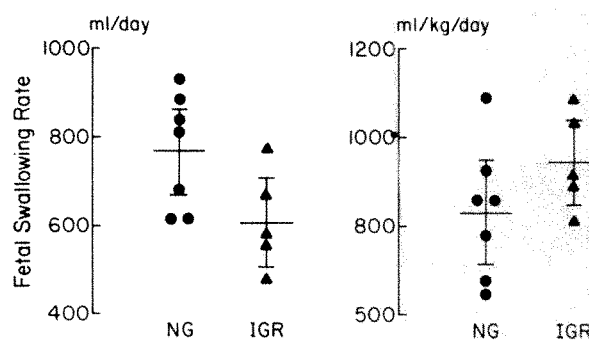


Fig. 2. Effect of intrauterine growth on estimated fetal swallowing rate of amniotic fluid at term. Mean $\pm$ SE are superimposed on a plot of individual values.

eighth day of pregnancy to term. Baboons were given nothing by mouth for 8 hours before each study and were observed to feed poorly for the following 48 hours. None of the dams had high blood pressure levels before, during, or after the pregnancy.

Results obtained in pregnancies with normally developed and growth-retarded fetuses were compared by means of Student's t test of the means.

Results

Results of amniotic fluid volume estimates are shown in Fig. 1. Although growth-retarded fetuses had mean ($\pm$ SE) amniotic fluid volumes that were 28% lower than those of their normally developed peers (320 ± 58.4 ml versus 445 ± 60.1 ml), the difference was not statistically significant. Expressed in milliliters per kilogram of fetal body weight, the means were quite similar (490 ± 66.9 ml/kg versus 475 ± 59.1 ml/kg).

Estimates of fetal swallowing rates are depicted in Fig. 2. Intrauterine growth-retarded fetuses appeared to ingest their amniotic fluid somewhat more slowly than normally developed fetuses (609 ± 50.9 ml/day versus 769 ± 48.6 ml/day, $p = 0.05$). The difference disappeared when the estimates were corrected for fetal body weight (945 ± 46.8 ml/kg/day versus 831 ± 57.7 ml/kg/day).

As shown in Table I, the average chemical composition of amniotic fluids from growth-retarded and normally developed fetuses were quite similar, except for pH which was significantly lower in growth-retarded fetuses (7.50 ± 0.083 versus 7.85 ± 0.084 , $p = 0.023$).

Comment

Our data suggest that the immediate environment of the baboon fetus at term is little affected by events that decrease its rate of growth. A possible exception might be maternal hypertension, an event this study did not address although it is a major cause of intrauterine growth retardation in human pregnancies. Volume and

Table I. Volume and chemical composition of amniotic fluid from baboons at term with normally developed and growth-retarded fetuses (mean $\pm$ SE)

Characteristic	Normally developed (n = 7)	Growth-retarded (n = 5)
Volume		
In milliliters	445 $\pm$ 60.1	320 $\pm$ 58.4
In milliliters per kilogram	475 $\pm$ 59.1	490 $\pm$ 66.9
pH	7.85 $\pm$ 0.084*	7.50 $\pm$ 0.083*
Osmolality (mosm/kg)	260 $\pm$ 6.8	266 $\pm$ 4.9
Proteins (gm/dl)	0.60 $\pm$ 0.175	0.45 $\pm$ 0.044
Creatinine ratio (amniotic fluid/maternal plasma)	1.77 $\pm$ 0.171	1.86 $\pm$ 0.288
Delta optical density at 450 nm	0 $\pm$ 0	0.004 $\pm$ 0.0028

*p = 0.023.

composition of amniotic fluid are mostly similar in pregnancies producing growth-retarded and normally developed babies. A major exception is the pH of amniotic fluid, which is less alkaline when the fetus is growth retarded. This might be a reflection of fetal acidemia, since the fetus exerts the predominant influence on amniotic fluid PCO_2 and pH.¹² There is evidence supporting the existence of a state of hypoxemia and acidosis in growth-retarded fetuses. Sheep fetuses with growth retardation induced by gradual embolization of the uteroplacental vascular bed show a decrease in mean PaO_2 .¹³ There is also indirect evidence in humans. Body water estimates obtained within 6 hours of birth show that growth-retarded neonates have excessive amounts of body water and that an excessive proportion of that water is located inside the cells.¹⁴ This cellular overhydration is corrected within a few hours of birth. Concurrent acid-base data are unfortunately not available. These findings may be explained by an impairment of cellular metabolism. Hypoxia and acidosis interfere with the production of adenosine triphosphate needed to power the pumps in cell membranes that keep sodium and water out of the cell and potassium inside. A malfunction of these pumps would result in an influx of water into the cells—a rapidly corrected event after birth when the neonate finds himself in a more favorable environment.

A decade ago investigators showed a great deal of interest in fetal ingestion of amniotic fluid, its proteins, and amino acids¹⁵ and in the possibility of correcting fetal growth retardation in utero by injecting amino acids into the amniotic cavity.^{16,17} One of the many practical questions that have remained unsolved is whether the amniotic fluid ingestion process was in any way impaired in growth-retarded fetuses. In light of this, it is interesting to note that our mean estimate of fetal swallowing rate of amniotic fluid was 21% lower in growth-retarded fetuses. When this estimate was corrected for fetal weight, however, the difference disappeared. In fact, growth-retarded fetuses swallowed (in milliliters per kilogram per day) an average of 14%

more amniotic fluid than their normally developed peers, but this difference was not statistically significant. One can also calculate that mean protein intakes were identical for growth-retarded and normal fetuses (5 ± 0.9 g/kg/day). This would seem to indicate that the slower fetal growth was not related to an insufficient supply of fluids and proteins via the amniotic route. It is an interesting point, since fetuses that either do not swallow (for instance, anencephalic fetuses) or cannot absorb any amniotic fluid (for instance, fetuses with duodenal atresia) are almost invariably growth retarded.

Whether our data may be extrapolated to human pregnancies with fetal growth retardation or to growth retardation related to maternal hypertension remains speculative. We have shown previously that amniotic fluid composition,¹ volume, and fetal ingestion rate² are quite similar in normal human and baboon pregnancies. It is likely therefore that the analogy may be extended to pathologic pregnancies as well. Resolution of this question must await more comparative studies.

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Orientation of elastic fibers in the human cervix

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We have determined the microanatomy of the cervix in relation to elastic fibers by serial dissection of human cervix in three tissue planes: cross, sagittal, and frontal sections. Analysis suggests that elastin is localized to specific regions of the uterine cervix and not dispersed throughout the connective tissue stroma. By Musto stain the majority of elastic fibers are noted to be oriented from the external os to the periphery and from there in a band upward toward the internal os where they become sparse in the area of the cervix with the greatest amount of smooth muscle just below the internal os. Elastic fibers were noted to be sparsely distributed in the cervical stroma. (*AM J OBSTET GYNECOL* 1986;155:219-24.)

Key words: Uterine cervix, elastin, elastic fibers

Normally the cervix shows considerable elasticity during gestation and parturition and undergoes profound changes altering the morphologic and biochemical makeup of the organ.¹⁻⁷

We previously reported the presence of elastin in the cervix.⁸ The amount of elastin present was found to be 1.54% to 1.59% of the connective tissue fraction of whole cervices,⁹ based on the relative proportion of the elastin-specific cross-links, desmosine and isodesmosine. However, the morphology of elastic fibers of the cervix has not been well elucidated.

The present study was conducted to determine the anatomic orientation of the elastic fibers in human cervical tissue and their relationship to connective tissue stroma to provide a theoretical basis for the role of cervical elastin throughout gestation and in dilatation

and effacement at parturition. The theory could then be validated by further experimentation.

Elastin functions in flexible connective tissue in a manner similar to polymeric rubber, which is viscoelastic. Elastin is not self-lubricating but requires the presence of water for its rubberlike properties to become apparent. It is found in tissues under continued stress such as skin and arteries and in tissues such as lung where cyclic deformation with minimal energy loss resulting from hysteresis is important.¹⁰

Material and methods

Tissue sources. Seven human uterine and cervical specimens were obtained fresh in the operating suite immediately following hysterectomy for benign gynecologic disease. The samples were obtained from premenopausal women who had normal menstrual cycles before surgery (see Table I for details).

Tissue preparation and staining for light microscopy. Each cervix was amputated from the uterine specimen by sharp dissection at the level of the internal os. Each cervix was then opened on the ventral surface from the external os to the internal os as shown in Fig.

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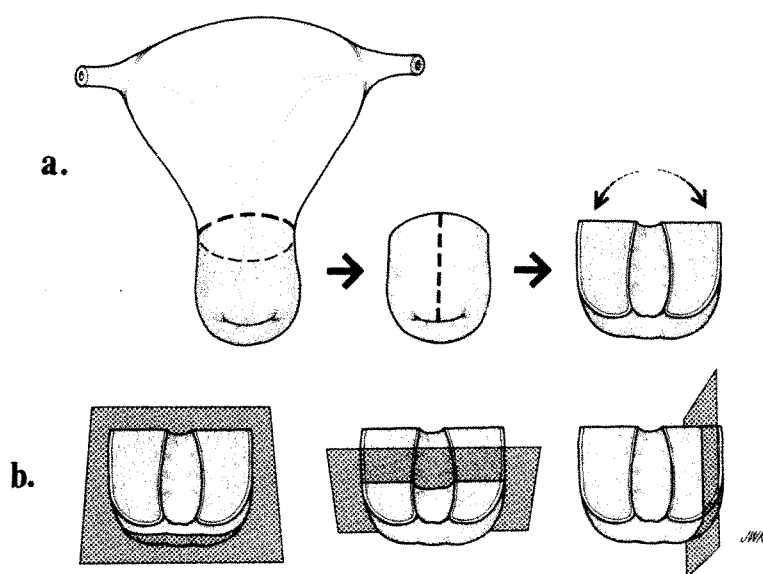


Fig. 1. *a*, Diagram of the cervical dissection. Dotted line indicates level of cervical amputation from the uterus and the opening of the cervix. *b*, Diagram indicating the three planes of dissection: frontal, cross, and sagittal sections.

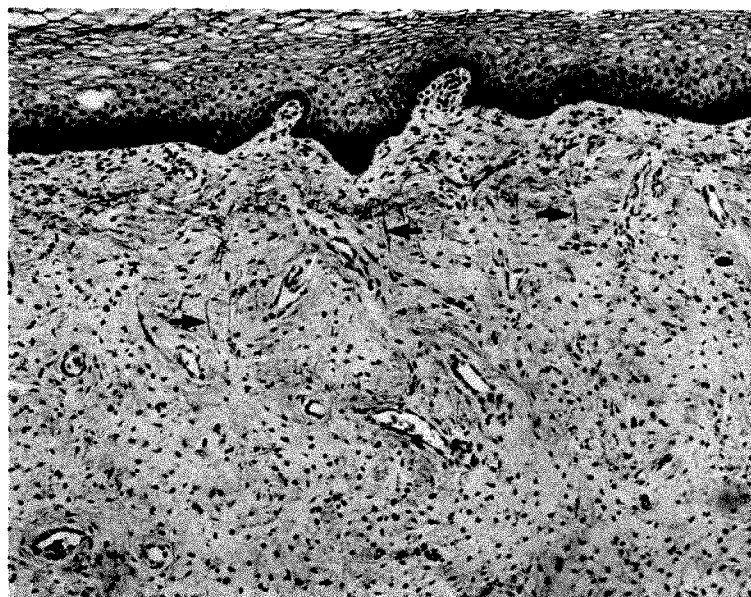


Fig. 2. Photomicrograph of a cross section of the cervical mucosa and stroma at the level of the external os. Note the presence of elastic fibers (arrows) beneath the mucosa and extending into the stroma. ($\times 130$.)

1, *a*. The tissue was then opened and pinned to a dissecting board. Two cervices, samples 1 and 6, were dissected horizontally, and samples 2, 3, 4, and 5 were dissected longitudinally along the endocervical canal and parallel to it. These tissues were pinned to a rubber stopper to keep the edges from becoming disoriented and were rolled and immediately placed in phosphate-buffered formalin. The tissue was processed routinely and embedded in Paraplast Plus. Serial sections were

prepared so that three planes—cross, sagittal, and frontal—would be represented (Fig. 1, *b*). These tissue sections were 6 μ m thick and were stained by an iron-hematoxylin stain described by Musto,¹¹ which reveals sharply outlined elastic fibers. Briefly, paraffin was removed from the tissues, and they were hydrated. They were mordanted in alcoholic saturated Bouin's solution at 55 to 60° C for 10 minutes. Sections were then washed in tap water, rinsed briefly in distilled water,

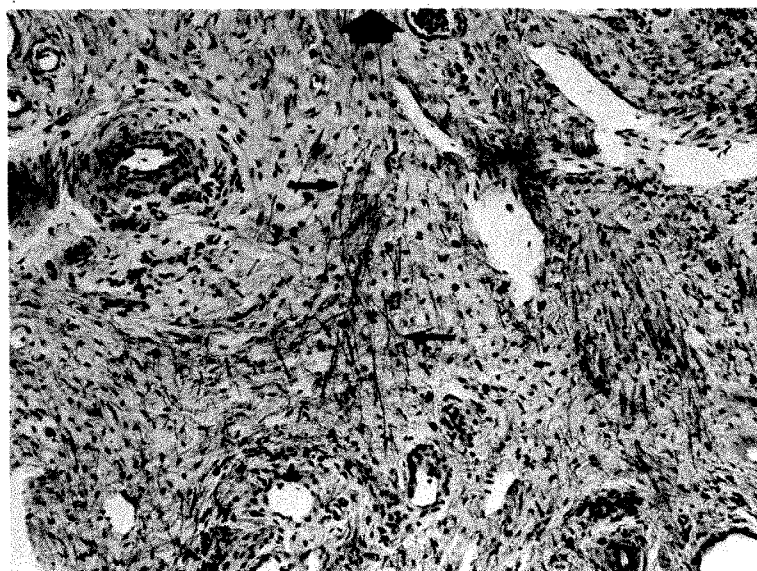


Fig. 3. Photomicrograph of a cross section of the deep cervical stroma at the level of the external os. Arrows indicate elastic fibers. Arrowhead indicates the direction of the mucosa. ($\times 260$.)

Table I. Tissue sources

Specimen No.	Age (yr)	Gravida	Para	Reason for removal of uterus
1	53	3	3	Fibroid uterus
2	45	3	3	Poor pelvic support
3	49	0	0	Intramenstrual bleeding
4	38	6	4	Fibroid uterus
5	44	0	0	Adhesions, chronic pelvic pain
6	34	0	0	Fibroid uterus
7	30	0	0	Fibroid uterus

and stained in Musto elastic stain for 45 minutes. After staining, the sections were washed in tap water, rinsed in distilled water, and stained in wood-stain scarlet for 5 minutes. After a second water wash the sections were treated with 2% phosphotungstic acid (2 minutes) and with 5% acetic acid (5 minutes), rinsed in distilled water, dehydrated in alcohol, and then stained in 1% alcoholic saffron (5 minutes). Sections were then dehydrated and cleared before mounting. Using this stain the elastic fibers stain black and are sharply outlined, nuclei stain dark purple to black, muscle cells stain pale red, and collagen stains yellow.

Results

Cross sections of specimens taken from the level of the external os revealed elastic fibers that run just below the mucosa (Fig. 2). These fibers then course downward from the mucosa into the deeper layers of the stroma just under the epithelium of the endocervical canal and portio vaginalis (Fig. 3). Reconstructions of several samples indicate that these fibers run as a group

at this level. Cross sections slightly more superior to the level of the external os reveal at that level that these elastic fibers can be found only in this layer of the stroma. The area under the mucosal lining and the middle layers of the stroma are free of fibers superior to the external os. Sections cut midway between the external and internal os reveal elastic fibers only in these areas of the stroma nearest the endocervical canal and portio vaginalis. These fibers continue upward for only a short distance, disappearing before reaching the internal os.

Sagittal sections taken from blocks starting at the external os reveal that there is a band of elastic fibers at this level of the stroma. These fibers run parallel to a plane drawn from the external os to the internal os. This band is 20 to 30 μm thick and contains elastic fibers running parallel to and between layers of collagen fibers (Fig. 4). The remainder of the stroma appears to be relatively free of elastic fibers. Sections taken at more superior levels reveal the continuation of this band until it disappears before reaching the internal os.

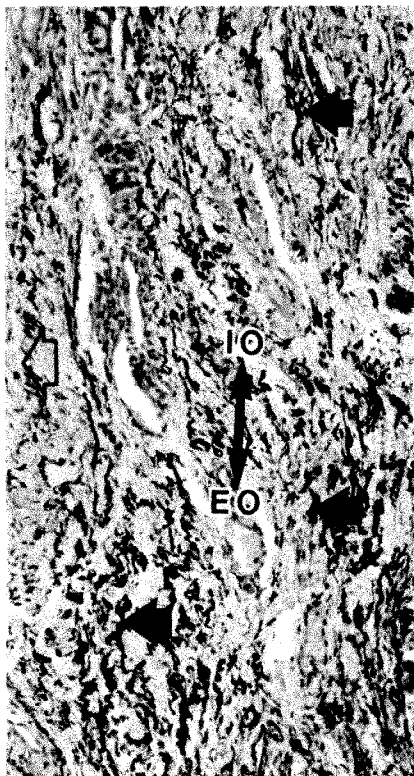


Fig. 4. Photomicrograph of a sagittal section of the deep portions of the stroma. Note the presence of elastic fibers (*arrowheads*) which run in a cephalic-caudal direction (*arrow*); *EO* and *IO* indicate the direction of the external and internal os, respectively. *Open arrowhead* indicates direction of the mucosa. ($\times 300$.)

Frontal sections of specimens taken at the level of the external os reveal elastic fibers in bands only at the deepest levels of the stroma nearest the endocervix and the portio vaginalis. These fibers are 2 to 4 μm wide and appear to run between bundles of collagen fibers (Fig. 5). Frontal sections in other regions of the stroma do not contain any fibers at this level. Sections slightly more superior contain approximately the same distribution of elastic fibers. Sections cut from blocks midway to the internal os demonstrate a continuation of this band of elastic fibers, which disappears before reaching the internal os.

The majority of the elastic fibers were observed to be oriented from the periphery of the vaginal portion of the cervix toward the external os and from there in a band upward to just below the internal os. At this junction the elastic fibers become sparse. The greatest amount of smooth muscle is just below the internal os and smooth muscle tapers off toward the external os. The results of this study demonstrate for the first time that elastin is localized to specific regions of the cervix and is not dispersed throughout the stroma (Fig. 6). This orientation was similar in all specimens, indicating

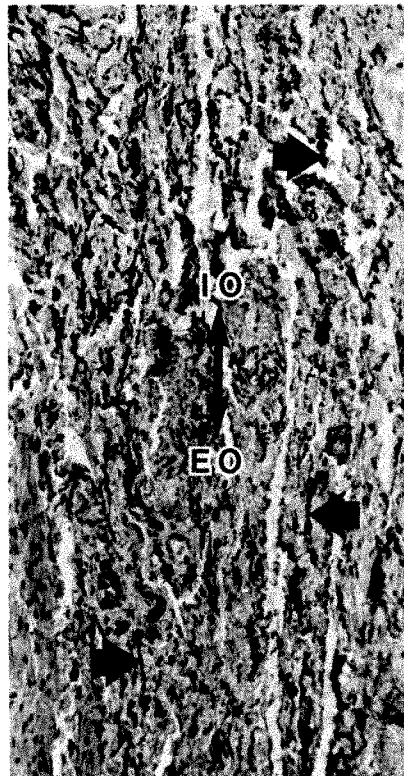


Fig. 5. Photomicrograph of a frontal section of the deep portions of the stroma. Elastic fibers (*arrowheads*) in this portion of cervix are orientated in a cephalic-caudal direction (*arrow*); *EO* and *IO* indicate the direction of the external and internal os, respectively. ($\times 300$.)

that age and parity did not affect this spatial relationship.

Comment

Danforth^{1,2} originally reported elastic fibers in the cervix stained with elastic and orcein-Metamine yellow stain, but they were not thought to be significant. He found them sparse and haphazardly arranged. Because of the small amount of tissue that took up these non-specific stains, elastin was not considered a constituent of the cervix. Pinto et al.¹² observed that elastic fibers in the human cervix appeared to be more disorganized and separated after intravenous treatment with estradiol-17 β . In their studies they used a Gomori paraldehyde fuchsin stain, which also is a nonspecific stain, thus the exact orientation of the fibers was not elucidated. Their findings, however, are of considerable interest because serum estradiol-17 β is increased during gestation. The stain used here is a modification of the original Verhoeff method¹³ and its modification.¹⁴ Verhoeff's modified method required differentiation and produced a background that was dark, not clear, and almost impossible to photograph.¹¹ The method used

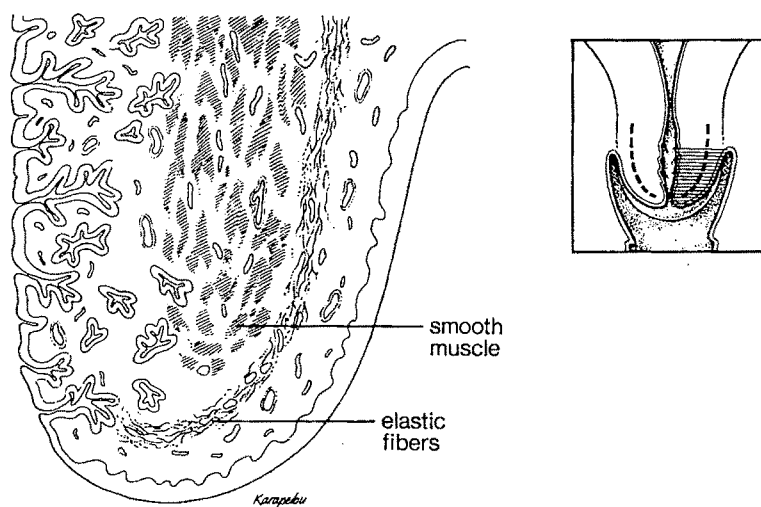


Fig. 6. Diagram of the distribution of elastic fibers in the cervix. Note that elastic fibers are located beneath layers of smooth muscle cells. *Insert* indicates region of the cervix represented in diagram.

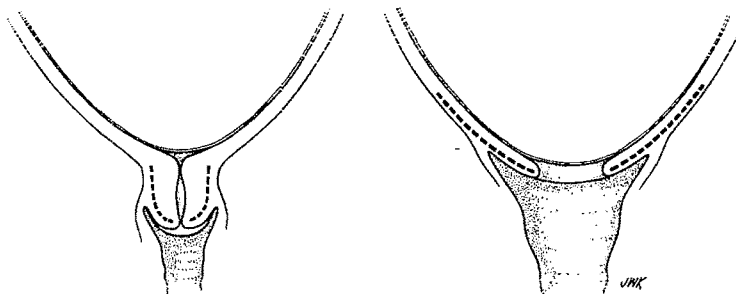


Fig. 7. Diagram representing effacement of the cervix showing orientation of elastic fibers. (Drawing modified from Danforth DN. The morphology of the human cervix. Clin Obstet Gynecol 1983;26:11.)

here does not require differentiation of elastic fibers and thus the time the sections remain in the elastic stain solution determines the final color. The elastic fibers are therefore more sharply outlined and muscle and collagen more lightly stained and better differentiated. Thus this new staining technique enabled us to locate the elastic fibers readily.

The observation that the organization of the elastic fibers of the human cervix is a band from the periphery to the external os and upward toward the internal os rather than a random diffuse dispersal throughout the stroma supports a role for cervical elastin during gestation and in dilatation during parturition.

After effacement this band of elastic fibers would be in the efficacious position for efficient dilation (Fig. 7).

The fibers are oriented in such a manner that they would retract upward and inward during effacement. When the cervix was completely effaced, the elastic fibers would be directly under the presenting part of the fetus. As this presenting part is pushed against the cervix during uterine contractions in active labor the

elastin stretches, eventually stretching to double its length. In the lung elastin serves as a framework for smooth muscles to contract against,¹⁵ and this could be one additional physiologic function for elastin in the cervix. Also a further function of mature cross-linked elastin oriented in the manner described here could include that of keeping the cervix closed until labor starts.

Likewise, the elastic fibers are in the correct orientation to allow for the resumption of the shape of the cervix when recoiling resumes at delivery. Although the cervix is quite soft and stretchy following delivery, it does begin to resume its prior shape fairly rapidly.

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The rate of increase of serum human chorionic gonadotropin in normal intrauterine pregnancy

To the Editors:

We read with great interest the report by Pittaway et al. entitled "Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies" (AM J OBSTET GYNECOL 1985;152:299).

It is undeniable that the rate of increase of serum human chorionic gonadotropin (hCG) in early pregnancy brings to the obstetrician very useful information. This is specially true in cases at risk for ectopic pregnancy, in threatened abortion, multiple pregnancy, or suspected trophoblastic disease, or simply when the last menstrual period is not reliable. The equation proposed by Pittaway et al. to describe the rate of increase of hCG fits much better with the reality of the experimental data than any of the doubling time coefficients previously published. However, for the clinician dealing with a suspected ectopic pregnancy the calculation of an expected mean increase, either from a doubling times coefficient or from a quadratic equation is not an easy task. Moreover, none of these methods gives the range that should be considered as a normal increase.

We like to present here a much easier way to predict the mean increase of serum hCG in early pregnancy. As reported by other investigators, we found that serum hCG has a log normal distribution. Therefore mean values and standard deviations have been calculated on a logarithmic basis. Surprisingly, however, when we tried various equations to represent the increase of hCG geometrical mean, we found that the

Table I. Rate of increase of serum hCG concentration in early normal intrauterine pregnancy (ng/ml)*

Human hCG concentration (ng/ml)	Gestational age (wk)		
	3	4	5-9
Range of the mean	<250	250-1000	>1000
Daily increase			
P ₉₀	73	210	1089
P ₅₀	73	100	517
P ₁₀	16	48	245

*Gestational age is expressed as completed weeks from last menstrual period.

best curve fitting from 5 to 9 weeks from last menstrual period (LMP) was obtained by the following simple linear regression:

Mean serum hCG (5 to 9 weeks) = gestational age (in days from LMP) × 517 - 17143 ng/ml.

As shown in Fig. 1, that regression line fits very closely with the geometric means of the experimental data. For ease of use we suggest that the following rounded value be kept in mind: from 5 to 9 weeks from LMP the mean hCG shows a daily increase of 500 ng/ml.

The tenth and ninetieth percentiles of hCG increase are probably more suitable parameters than the mean increase, since they can be used as arbitrary limits between normal and abnormal evolution. For instance, an increase of serum hCG of <250 ng/ml/day (P₁₀ in Fig. 1) should suggest the possibility of ectopic pregnancy or abortion.

The above equation does not fit with the data obtained before day 35, and therefore other criteria should be used for the end of the cycle and the first week after the missing period.

The rates of increase of serum hCG in early normal intrauterine pregnancy are presented in Table I.

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Reply

To the Editors:

It was not the intent of our report to provide methods to evaluate the normalcy of early pregnancy but to describe the nonlinearity of the exponential rise of serum hCG in normal pregnancy before the sixth to seventh week of gestation. In another report¹ we have recom-

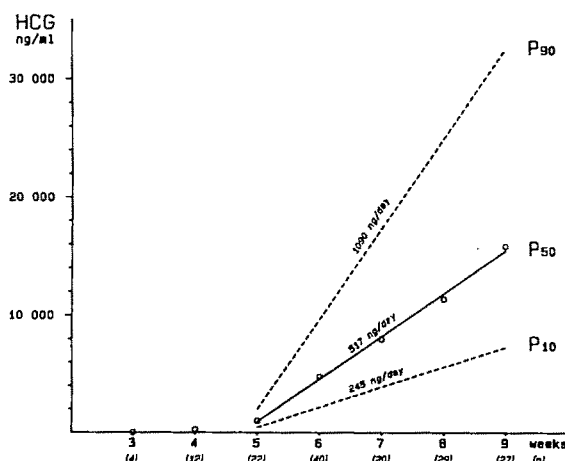


Fig. 1. Serum hCG concentration in normal intrauterine pregnancy. Gestational age is expressed as completed weeks from last menstrual period. Geometric means of experimental data are represented by the open circles and the linear regression of these means by the solid line. The upper (P₉₀) and lower (P₁₀) limits of the normal range are also shown (interrupted lines). The number of samples measured in each group is indicated in parentheses.

mended use of the 85% upper confidence limits of the hCG-doubling time, which for days 23 to 35 or sampling range 1 to 200 ng/ml and for days 35 to 42 or sampling range 200 to 1000 ng/ml were determined to be 2.2 days and 2.6 days, respectively. With use of a scientific calculator with a log scale, the determinations of doubling time are quite simple by means of the following formula:

$$\text{Doubling time} = \frac{(\log 2) \times (\text{interval in days})}{\log \frac{[\text{hCG}_2]}{[\text{hCG}_1]}}$$

Beyond 5½ to six weeks of gestation, the combination of serum hCG and sonography is very efficacious in determining normal pregnancies.²

The method proposed by Schwerts et al. is easier and simpler to use and is similar to previous recommendation based on a linear rise of the log of hCG concentrations.³ However, the method requires that the first day of the last menses be known. In addition, the linear regression has been calculated for the fifth to ninth week when significant variability of serum hCG occurs, the serum hCG of some normal pregnancies will be reaching a plateau, and sonography becomes the more important technique for evaluation. When I applied the proposed method retrospectively to 13 known ectopic pregnancies that were all diagnosed before tubal rupture, the method could not be used for six (one the last menstrual period was unknown and five were less than the fifth week of gestation). Of the remaining seven, three had a normal rise and four had an abnormal rise by the proposed criteria. So in my own experience in an infertility practice, the usefulness of the proposed method in the early diagnosis of ectopic pregnancy is limited.

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3. Kadar N, Caldwell BV, Romero R. A method of screening for ectopic pregnancy and its indications. *Obstet Gynecol* 1981;58:162.

Thin-layer chromatography and false positive identification of phosphatidylglycerol in patients with preterm ruptured membranes

To the Editors:

We read with interest the study of patients with preterm rupture of the membranes (AM J OBSTET GYNECOL 1985;153:147) reported by Drs. Van Dorsten,

Horger, and Miller. The authors expressed disappointment in the predictive value of vaginal pool phosphatidylglycerol studies. They theorized that one-dimensional thin-layer chromatography might not be appropriate for amniotic fluid collected from the vagina. We heartily agree.

When originally constructing the methodology for a study reported 5 years ago (Stedman CM, Crawford S, Staten E, Cherny WB. AM J OBSTET GYNECOL 1981; 140:34-8), we too were tempted to use one-dimensional thin-layer chromatography. However, preliminary samples were difficult to interpret confidently with use of the one-dimensional technique, because of suprious densities that were frequently present near the level of the phosphatidylglycerol standard. When a two-dimensional technique was applied, the true phosphatidylglycerol spot, if present, clearly separated from the other densities. Because of the potential adverse perinatal consequences of a clinician acting on a false positive test, the two-dimensional technique was chosen.

It is also necessary to point out that Van Dorsten et al. erroneously stated in their report that "Stedman et al. found that a vaginal pool L/S ratio of ≥ 2 also precluded RDS." Our report only referred to phosphatidylglycerol results and did not list the lecithin/sphingomyelin ratio for any patient.

Like the authors, we eagerly await the results of an ongoing prospective study to test the relative merits of one-dimensional versus two-dimensional chromatography. We expect they will find the two-dimensional technique to be superior and suggest that trace phosphatidylglycerol results be considered clinically the same as negative.

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Scott Crawford, M.D.

Walter B. Cherny, M.D., C.M.

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Reply

To the Editors:

We appreciate the comments of Stedman, Crawford, and Cherny. Since the article's publication, I have had a number of people confirm the vagaries of one-dimensional thin-layer chromatography. Unfortunately, we have not yet looked at the data in our prospective study comparing the different techniques for phospholipid analysis. We certainly hope to confirm the reliability of the two-dimensional technique, since vaginal pool phospholipid analysis has become an important tool in our procedures.

The authors regret that they inappropriately ascribed a quote to your study; the quote should have been attributed to Brame and MacKenna (Vaginal pool

phospholipids in the management of premature rupture of membranes. *AM J OBSTET GYNECOL* 1983; 145:992).

J. P. Van Dorsten, M.D.
E. O. Horger, M.D.

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Concern about saline amnioinfusion

To the Editors:

I read with interest the article on saline amnioinfusion by Miyazaki and Nevarez in October 1, 1985 (*AM J OBSTET GYNECOL* 1985;153:301-6). In the article they reported relief of variable decelerations with amnioinfusion of normal saline solution during the first stage of labor.

I am concerned about their report of a prolapsed umbilical cord 2 hours after performing the procedure. It raises the question whether this procedure increases the incidence of umbilical cord prolapse and also whether this particular infant would not have done better with more conventional forms of therapy for repetitive variable decelerations in the first stage of labor.

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Reply

To the Editors:

Since termination of the study in March, 1984, saline amnioinfusion has become a routine procedure. There have been no additional instances of cord prolapse in over 400 amnioinfusions (1:400). The usually quoted incidence of cord prolapse in vertex presentation is 1:200.

The conclusions are obvious: no causal relationship.

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Wiping routine: No problem in natural method

To the Editors:

I wish to respond to Dr. Charles Norris's letter regarding negative effects of wiping in the use of the ovulation method, Creighton Model (*AM J OBSTET GYNECOL* 1985;152:1108-9). He claimed that his patients using this technique of observation suffered irritation and itching as a result of the frequent wiping. As an instructor in the Creighton style, I have never had a client who has had an irritation or itch as a direct result of the wiping routine. Those who have had such prob-

lems seem to be able to get rid of them by eliminating certain products, such as scented tampons or pads, bubble bath, detergents, dryer fabric softeners, or douches. Sometimes the client needs to stop wearing tight jeans or start buying panty hose with a ventilated crotch. If there is a discharge, the physician may find something that needs treatment and that is a different matter. But through all this, the woman can continue her observational routine as long as she has been taught, correctly, to wipe gently from front to back with a thick pad of folded toilet tissue.

Again, I have never had clients with the problem described by Dr. Norris and I have been teaching the ovulation method for 10 years, five in the Creighton Model. I have found most of the women to be remarkably free of any vulvar or vaginal irritation on the whole.

I hope other Creighton Model teachers of natural family planning will also write about this letter because I feel that Dr. Norris was really reaching this time. His claim is simply unfounded.

Molly Woerner, C.N.F.P.P.

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Reply

To the Editors:

Molly Woerner, in her defense of the Creighton Model observational routine, misquotes me. My letter does not refer to an "itch," but rather an irritation in 25.9% of the clientele I instructed in the method. It is specifically an irritation upon repeated wiping which many women are reluctant to mention out of concern that they are the only ones experiencing this discomfort. The irritation can also persist.

At least 20% of the population is genetically atopic,¹ and the incidence seems to be increasing (Hanifin J. M., Department of Dermatology, University of Oregon Health Sciences Center, personal communication). Furthermore, approximately 33% of all patients evaluated by dermatologists are afflicted with a form of eczematous dermatitis.² Genetically atopic individuals are at an increased risk of developing an irritation when the interlabial sulcus of the vulva is repeatedly rubbed with an abrasive substance. Although products such as scented tampons can also cause problems of this nature, the excessive wiping of the vulva, alone, can cause multiple microscopic abrasions to these delicate tissues which, in turn, lessen resistance and further predispose the client, particularly the atopic individual, to untoward tissue reactions which she otherwise may well be resistant to. This is a problem in basic dermatology, not in natural family planning and not in gynecology.

The pathophysiology is somewhat similar to that of a person repeatedly blowing his nose or sneezing into facial tissue during a cold. Great numbers of microscopic pulp spicules are inhaled and impaled on the mucus membranes of the nares, setting up an intense inflammatory response for all to see.

While the Creighton Model observational routine is an excellent research tool, the numbers reported in my original letter (August 15, 1985) would seem to indicate that this observational routine is suitable for only 60% to 70% of the general population.

The matter remains to be studied scientifically.

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REFERENCES

1. Lynch PJ. Dermatology for the house officer. Baltimore: Williams & Wilkins, 1982:188.
2. Fitzpatrick TB, et al. Dermatology in general medicine. 2nd ed. New York: McGraw-Hill, 1979:507.

Symphysiotomy for shoulder dystocia

To the Editors:

Symphysiotomy was mentioned as a mode of treatment for shoulder dystocia in Sandberg's article about the Zavanelli maneuver (AM J OBSTET GYNECOL 1985; 152:479-84). Drs. Hibbard, Weber, and McCorriston all mention symphysiotomy in the discussion of the paper. However, the participants did not have personal experience with the operation.

I wish to confirm that subcutaneous symphysiotomy can be completed within 5 minutes with the only equipment required being a urethral catheter and scalpel. The symphyseal joint is soft and easily divided in pregnancy. With a 2.5 cm separation of the pubic bones, sufficient room should be available to deliver the impacted shoulders in nearly every case.

However, there is need for caution in using this simple operation. Symphysiotomy has remained outside most people's experience, not because it is a bad operation in itself but because it is easily abused. This has happened in the past, but careful limitation of separation to under 3 cm will keep complications to a minimum.^{1,2} Maternal mortality will always be less than that of cesarean section.

The danger in the emergency situation of shoulder dystocia is trauma to the soft tissues in the area of the urethra and anterior vaginal wall, which are left relatively unprotected and placed on tension by the symphyseal separation. A large episiotomy, backward displacement of the fetus, and gentle delivery of the baby will minimize maternal trauma. The latter is the hardest maternal safeguard to achieve in a desperate and stressful emergency situation.

The risk of maternal soft tissue trauma has to be weighed against the almost certain loss of the baby if other methods of vaginal delivery are proving unsuccessful, as well as the considerable maternal soft tissue trauma and infection likely with the technique of replacement of the baby into the vagina, followed by cesarean section.

Subcutaneous symphysiotomy should remain one of the treatment options in severe shoulder dystocia, and it could well be the best option. However, it can never be a practical or safe option unless it is taught to obstetricians. For most people at present it is little more than a name with an unenviable reputation.

Its place as an alternative to cesarean section in the treatment of moderate cephalopelvic disproportion needs to be assessed in the United States. Should this happen and the operation found to be useful, the experience achieved with it in simpler and less stressful situations would enhance its safety and effectiveness in the acute and rare emergency that ensues when the shoulders impact.

V. J. Hartfield

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REFERENCES

1. Hartfield VJ. Subcutaneous symphysiotomy—time for a reappraisal? Aust NZ J Obstet Gynaecol 1973;13:147-52.
2. Hartfield VJ. A comparison of the early and late effects of subcutaneous symphysiotomy and lower segment cesarean section. J Obstet Gynaecol Br Commonw 1973;80:508-14.

Capnocytophaga on the fetal surface of the placenta of a patient with ruptured membranes at 39 weeks' gestation

To the Editors:

I had a similar case to the one reported by Ernest and Wasilaukas on *Capnocytophaga* in amniotic fluid (Ernest JM, Wasilaukas B. *Capnocytophaga* in the amniotic fluid of a woman in preterm labor with intact membranes. AM J OBSTET GYNECOL 1985;153:648). My patient was admitted to the hospital at 39 weeks' gestation with spontaneous rupture of membranes for 30 minutes. She also had active recurrent vulvar herpes lesions.

Other than her fever of 38.3° C and her vulvar lesions, there were no signs or symptoms of infection. Cultures from the fetal surface of the placenta, at cesarean section, were positive for *Capnocytophaga* and placenta pathologic studies showed acute amnionitis. The amniotic fluid was neither cloudy nor foul smelling.

The patient was given intravenous cefazolin sodium after delivery with a good response. The 3820 gm male infant, with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively, was not treated with antibiotics and never developed evidence of infection.

Another case report of premature labor with intact membranes and *Capnocytophaga* amnionitis¹ is noted to be similar to that reported by Ernest and Wasilaukas.

The absence of mention of this apparently pathogenic organism in the obstetrics and gynecology literature before 1985 is particularly interesting. Perhaps it has been overlooked as a pathogen that can penetrate

intact membranes and may be an etiologic agent of premature labor.

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REFERENCE

1. Mercer LJ. *Cepnocytophaga* isolated from the endometrium as a cause of neonatal sepsis: a case report. *J Reprod Med* 1985;30:67.

Abdominal pelvic pain syndrome: Fact or fiction

To the Editors:

We read with interest the article on the abdominal pelvic pain syndrome D. G. Slocumb (*AM J OBSTET GYNECOL* 1984;149:536-43) and the subsequent correspondence (*AM J OBSTET GYNECOL* 1985;151:701-2).

Our recently published study¹ of pelvic venography in women with chronic pelvic pain without obvious organic cause shows beyond doubt that pelvic varicosities are present in the majority of these women. A venogram score of ≥ 5 clearly distinguishes women with pelvic pain syndrome from those who have an "organic" cause or no pelvic pain (diagnostic sensitivity, 91%, and specificity, 89%). Abdominal pressure over the "ovarian point" (Fig. 1) commonly reproduces the pain complained of, especially on the right side. Our pelvic

venography studies suggest that pressure over the ovarian point occludes the ovarian vein, and we have postulated that the resultant pain is due to the back pressure on the hilum of the ovary. We consider that pelvic pain has an organic as well as a psychological component and that the "fact or fiction" part of the title of Slocumb's article is no longer a valid question.

The treatment of this condition is not so clear. All pain must have a neurophysiologic component, but we find it difficult to accept that abdominal trigger points exist in women with the pelvic pain syndrome which can be anesthetized to provide long-term cure of the pain. It should be noted that Slocumb's study is uncontrolled. There is no placebo and no no-treatment controls in the design, and hence conclusions about the efficacy of trigger-point anesthesia in this group of patients must be limited. The presence of pelvic varicosities and the predominance of the ovary as the site of the pain along with the history of long-standing emotional disturbance tend to support Duncan Taylor's² concept of pelvic congestion as the somatic component of a psychosomatic disorder.

There can be no doubt of the very real need to establish an effective and easily applied therapy for this disabling condition. In view of the complex nature of pain experience it is likely that an effective treatment will only be achieved when a better understanding of the etiology of pelvic varicosities and psychological factors in pelvic pain has been acquired.

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REFERENCES

1. Beard RW, Highman JH, Pearce S, Reginald PW. Diagnosis of pelvic varicosities in women with chronic pelvic pain. *Lancet* 1984;2:946-9.
2. Duncan CH, Taylor HC. A psychosomatic study of pelvic congestion. *AM J OBSTET GYNECOL* 1952;64:1-12.

Attack rate for neonatal herpesvirus

To the Editors:

The letter by Hankins (*AM J OBSTET GYNECOL* 1985;153:816-7) in response to the letter by Smith et al. suggests that the attack rate for neonatal herpesvirus infection is 50%. Where is such a number taught? Under the most favorable circumstances experimentally, we were able to show an attack rate in mice of only 25%.¹ It is probably much less in humans.

I do not believe that the techniques of freezing or storing clinical specimens account for the differences

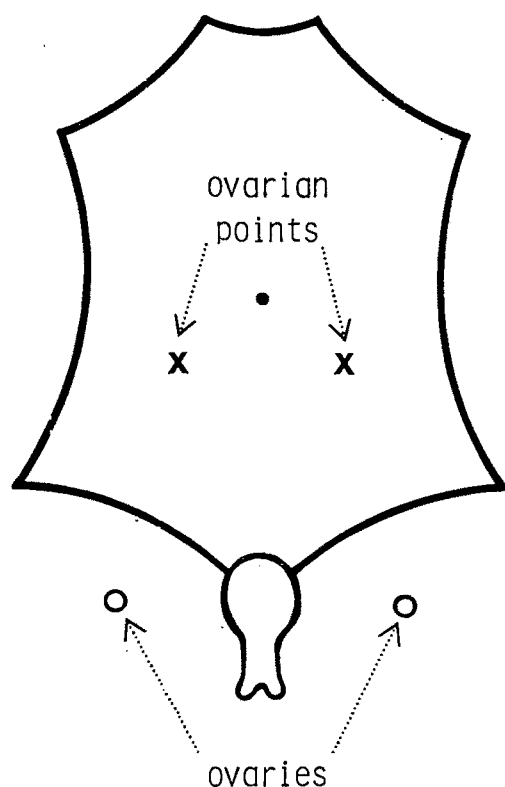


Fig. 1. Sites of tenderness.

in the studies cited by the two letters. Most probably all of the studies are looking at somewhat different populations. Neither the frequency of herpesvirus isolation rates from asymptomatic women nor laboratory techniques explain away the observation of infrequent neonatal herpesvirus infections in the face of relatively frequent maternal isolation of virus. Clearly there is some host response, immune or otherwise, that accounts for these differences, and we do not yet understand them.

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REFERENCE

1. Amstey MS, Kobos K. An experimental model for disseminated herpesvirus infection of the newborn. *AM J OBSTET GYNECOL* 1976;125:41.

Reply

To the Editors:

In our original report we stated many of the same concerns expressed by Dr. Amstey in his letter. Notably, that disparate recovery rate for herpesvirus exist depending on whether culture studies were made for women while in labor or in the third trimester but remote from labor. We also advanced the same hypothetical explanations for these disparate findings as did Dr. Amstey in his letter, that is, different patient populations, antibody present in cervical mucus and in amniotic fluid capable of neutralizing the virus, and potentially a dilutional effect from all these fluids and secretions washing past the cervix and reducing the viral inoculum below the critical amount required for a clinical infection.¹ In response to the letter by Smith et al.,² who incidentally confirmed our results but who proposed that we had used a viral recovery method capable of yielding false negative results, we were merely attempting to demonstrate that more sensitive tests might lead to unnecessary abdominal delivery of many of these infants. In essence we concur with Dr. Amstey that the attack rate for neonatal herpesvirus is low if one excludes primary infections. This would especially appear to be so with secondary infections and asymptomatic infections. Notwithstanding, let us remember that the majority of neonatal herpesvirus infections today occur in infants of women who were asymptomatic at delivery. As to the "classically" taught 50% attack rate, I would refer you to standard text books.^{3,4} In summary, we agree with all of Dr. Amstey's points and appreciate the opportunity to respond to one whom we view as a giant in this area.

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REFERENCES

1. Hankins GDV, Cunningham FG, Luby JP, et al. Asymptomatic genital excretion of herpes simplex virus during early labor. *AM J OBSTET GYNECOL* 1984;150:100-1.
2. Smith LH, Hepler JK, Chang RS. Asymptomatic herpes virus genital excretion during labor. *AM J OBSTET GYNECOL* 1985;153:815-6.
3. Sever JL, Larsen JW, Grossman JH. Handbook of perinatal infections. 1st ed. Boston: Little, Brown, 1979:11-21.

Conservative management of preeclampsia

To the Editors:

I read with some considerable alarm the article entitled "Maternal and perinatal outcome of conservative management of severe preeclampsia in midtrimester" (*AM J OBSTET GYNECOL* 1985;152:32-7). These results are so markedly different to the pregnancy outcome in a comparable group of patients published from my unit¹ that I thought some form of editorial comment may be worthwhile, since your JOURNAL is a very widely read publication. The article by Sibai et al. gives an unfair and perhaps inappropriate bias to the management of these patients. As you can see from our publication, these patients do remarkably well considering the severity of their disease and we certainly did not have the maternal morbidity and fetal loss reported by Sibai et al.

I would be interested in hearing your views with regard to this matter.

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REFERENCE

1. Horvath JS, Korda A, Child A, et al. Hypertension in pregnancy: a study of 142 women presenting before 32 weeks' gestation. *Med J Austr* 1985;143:19.

Reply

To the Editors:

I would like to thank Dr. Horvath for his interest in our report concerning the management of severe preeclampsia in midtrimester. He claims that the results of our study are markedly different from their outcome in a comparable group of patients. In this regard I think the two groups of patients are far from being comparable. He defines preeclampsia as a diastolic blood pressure of ≥ 85 mm Hg or a rise in diastolic of 15 mm Hg. Proteinuria was present in only 38 of 84 (45%) of his patients diagnosed as preeclamptic in Table I.¹ Yet, he does not define the quantity of proteinuria. Hence, most of his patients listed in Table I had mild to moderate pregnancy-induced hypertension and not severe

preeclampsia. The average systolic blood pressure in our patients was 179 ± 9 mm Hg, whereas the average diastolic pressure was 114 ± 8 mm Hg. In addition, the average 24-hour urinary excretion of protein was 5.3 ± 2.6 gm per 24 hours and the average serum uric acid was 7.9 ± 1.2 mg/dl. Moreover, 34 of them were referred to us from local physicians. So these patients are comparable to his 21 patients listed in column 2 in the first table. However, the average gestational age in the referred group in his series ($n = 21$) was 27 ± 2 weeks while the average gestational age of our patients was 24.3 ± 1.9 weeks, with 31 patients having a gestational age of <25 weeks and all of them having gestational age of <27 weeks at time of diagnosis. The perinatal mortality in the 21 patients reported by Dr. Horvath was 52%, which is higher than the perinatal mortality in 29 comparable patients in our report.

During the period of our report, about 1200 patients were encountered who were comparable to the 142 women reported by Dr. Horvath. These patients had hypertension in pregnancy before 32 weeks' gestation with 700 similar to their group in Table I and 500 similar to the group in Table II. The perinatal outcome in these patients was significantly lower than that reported by Dr. Horvath irrespective of the use of antihypertensive medications.

Finally, the review of the literature on page 36 of our report concerning outcome in similar groups of patients managed by others strongly argues against any potential benefit of antihypertensive therapy.

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REFERENCE

1. Horvath JS, Korda A, Child A, et al. Hypertension in pregnancy: a study of 142 women presenting before 32 weeks' gestation. *M J Aust* 1985;143:19.

Other aspects of the role of C-reactive protein during pregnancy and labor

To the Editors:

We were immensely interested to read Potkul et al.'s finding (The association of subclinical infection with preterm labor: the role of C-reactive protein. *AM J OBSTET GYNECOL* 1985;153:642-5) establishing the close correlation between preterm labor, subclinical infection, maternal serum C-reactive protein (CRP) level, and the outcome of tocolysis.

In our studies we measured the levels of CRP by enzyme-linked immunosorbent assay (reagents: Dako Ltd.) in 473 women with uncomplicated single pregnancies between gestational weeks 6 to 41. We have also

determined serum CRP concentrations in 60 healthy women in established labor with intact membranes as well as at 1 hour after delivery. Labor lasted not more than 12 hours in each case. Similarly, we made CRP determinations during preterm labor in six cases (gestational weeks 30 to 34) in which tocolysis was not attempted because of progress of labor.

According to our results the fiftieth centile of normal range during pregnancy is at 0.3 mg/dl and the ninetyth centile is at 1.0 to 1.2 mg/dl. Previously, in non-pregnant women lower values (mean $\pm$ SD: 0.15 ± 0.10 mg/dl) were found in our institute. The CRP values during labor and immediately after delivery were 0.80 ± 0.50 and 1.20 ± 0.60 mg/dl, respectively. Differences between these latter two values and also between the values found at the end of pregnancy and those found during labor were statistically significant ($p < 0.001$). Thus maternal CRP levels showed a rapid and significant increase during labor. A similar pattern of increase in CRP levels was found in six cases of preterm labor as well. With the background of these changes we doubt the direct effect of prostaglandins was exerted on hepatocytes, however.¹ We, rather, suppose there was a possible significant role of cellular immunologic events. The significant increase of natural killer-cell activity during labor has recently been shown.² Higher natural killer-cell activity was also found in cases of preterm labor.² These phenomena can be explained by the fall in progesterone level or the increase of prostaglandin production.³ Increased natural killer-cell activity is accompanied by the deliberation of cell-mediators, such as interleukin-1.⁴ This latter is known to induce CRP production in hepatocytes.

Thus, according to our data, increase of CRP level in maternal sera can also be provoked by merely regular uterine contractions, regardless of the origin of prostaglandin production whether being initiated by bacterial phospholipase-A₂ or any other factor.

In contrast with the findings of Potkul et al., according to our findings the upper limit of normal range of CRP level during pregnancy was not 0.7 mg/dl but higher (1.0 to 1.2 mg/dl). Taking these latter values into account we suppose that in cases of preterm labor, considering the outcome of tocolysis, the proportion of false positive cases (higher CRP level, successful tocolysis)—which was 37.5% in their report—could be diminished. Also, it would be worth repeating CRP determinations some hours later. In 15 of 60 term deliveries we found CRP levels <0.7 mg/dl during labor, but values >0.7 mg/dl were registered after delivery. That means that regular contractions of the uterus in well-established labor are accompanied by a significant increase of CRP production without any bacterial infection.

Based on these data we conclude that elevated maternal serum CRP level and/or significant increase of this value in pregnancy may well be an acute phase

Books received

- Alpha-Fetoprotein and Congenital Disorders.** Edited by Gerald J. Mizejewski and Ian H. Porter. 363 pages, illustrated. Orlando, Florida, 1985, Academic Press, Inc. \$35.00.
- Ambulatory Prenatal Nursing Care: Practice Standards for Positive Pregnancy Outcomes.** Marjorie R. Berg, LaVohn E. Josten, Karen Knoll, Barbara A. Taylor, and Marilyn K. Wilfrong. 204 pages. New York, 1986, Springer Publishing Company. \$21.95 (soft cover).
- Breastfeeding, Child Health and Child Spacing. Cross-cultural Perspectives.** Edited by Valerie Hull and Mayling Simpson. 216 pages. Dover, New Hampshire, 1985, Croom Helm Ltd. \$26.00.
- Breastfeeding Success for Working Mothers.** Marilyn Grams. 155 pages, illustrated. Sheridan, Wyoming, 1985, Achievement Press. \$15.00.
- Childbirth for Men.** Herbert Brant. 206 pages, illustrated. New York, 1985, Oxford University Press. \$7.95 (soft cover).
- Coming to Terms.** Roberta Israeloff. 172 pages. New York, 1985, Penguin Books. \$5.95 (soft cover).
- The Complete Guide to Pregnancy Testing and Counseling.** Updated, edited, and revised by Lori Saltzman and Michael S. Policar. 124 pages. San Francisco, 1985, Planned Parenthood. \$14.95.
- Diseases of the Breast.** Third Edition. Cushman D. Haagensen. 1072 pages, illustrated. Philadelphia, 1985, W. B. Saunders Company. \$90.00.
- Essential Management of Obstetric Emergencies.** Thomas F. Baskett. 221 pages, illustrated. Somerset, New Jersey, 1985, John Wiley & Sons, Inc. \$35.00.
- Feeding the Mother and Infant.** Myron Winick. 164 pages, illustrated. Somerset, New Jersey, 1985, John Wiley & Sons, Inc. \$49.95.
- First Trimester Fetal Diagnosis.** Edited by M. Fracaro, G. Simoni, and B. Brambati. 355 pages, illustrated. New York, 1985, Springer-Verlag New York, Inc. \$62.00.
- Gynecologic Oncology.** Robert C. Knapp and Ross S. Berkowitz. 658 pages, illustrated. New York, 1986, Macmillan Publishing Company. \$65.00.
- Immunology and Immunopathology of Reproduction.** (Contributions to Gynecology and Obstetrics, Vol. 14.) V. Toder and A. E. Beer. 212 pages, illustrated. New York, 1985, S. Karger. \$66.50.
- Implantation of the Human Embryo.** Edited by R. G. Edwards, Jean M. Purdy, and P. C. Steptoe. 459 pages, illustrated. Orlando, Florida, 1985, Academic Press, Inc. \$59.50.
- The Kidney in Pregnancy.** Edited by Vittorio E. Andreucci. 237 pages. Boston, 1985, Martinus Nijhoff. \$59.95.
- Making Miracles: In Vitro Fertilization.** Nan and Todd Tilton, Gaylen Moore. 200 pages. New York, 1985, Doubleday & Company. \$16.95.
- Manual of Neonatal Care.** Second Edition. Edited by John P. Cloherty and Ann R. Stark. 658 pages. Boston, 1985, Little Brown & Company. \$18.95 (soft cover).
- Obstetrics and Gynecology.** (The National Medical Series for Independent Study.) William W. Beck, Jr. 333 pages. New York, 1986, John Wiley & Sons, Inc. No price listed.
- Ovarian Cancer.** Edited by Norman M. Bleehen. 144 pages, illustrated. New York, 1985, Springer-Verlag New York, Inc. \$55.00.
- Pelvic Floor Anatomy and the Surgery of Pulsion Enterocoele.** Robert F. Zacharin. 170 pages, illustrated. New York, 1985, Springer-Verlag New York, Inc. \$45.00.
- Perinatal Care and Gestosis.** M. Suzuki and N. Furuhashi. 536 pages. New York, 1985, Elsevier Science Publishing Co., Inc. \$101.75.
- Proteins of the Placenta. Biochemistry, Biology and Clinical Application.** P. Bischof and A. Klopfer. 208 pages, illustrated. New York, 1985, S. Karger. \$76.25.
- A Question of Life.** Mary Warnock. 110 pages. New York, 1985, Basil Blackwell Inc. \$8.95 (soft cover).
- Richard Wesley TeLinde.** Howard W. Jones, Jr., Georgeanna Seegar Jones, and William E. Ticknor. 117 pages, illustrated. Baltimore, 1986, The Williams & Wilkins Company. No price listed.
- Vaccine Supply and Innovation.** Institute of Medicine. 224 pages. Washington, D. C., 1985, National Academy Press. \$21.00.

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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Specialty Review in Obstetrics and Gynecology: Practical Aspects, September 15-20, 1986, Chicago, Illinois. For additional information, contact the Registrar's Office. Tel.: In Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

A Clinical and Histopathologic Overview of Obstetrics & Gynecology, New York Hilton, New York, New York, October 11-16, 1986. For information contact: Department of Obstetrics and Gynecology, Saint Barnabas Medical Center, Livingston, NJ 07039.

Intensive Board Review Course in Reproductive Endocrinology, September 11-14, 1986, Massachusetts General Hospital, Boston, Massachusetts. Contact: Dr. William F. Crowley, Jr., Vincent Research Laboratories, Massachusetts General Hospital, Boston, MA 02114.

New Concepts in the Management of OB/GYN Infections, September 3, 4, and 5, 1986, Grand Hyatt, New York City. For further information contact: Selma Abdo, Continuing Education,

Upstate Medical Center, 766 Irving Ave., Syracuse, NY 13210. Tel.: (315) 473-4304.

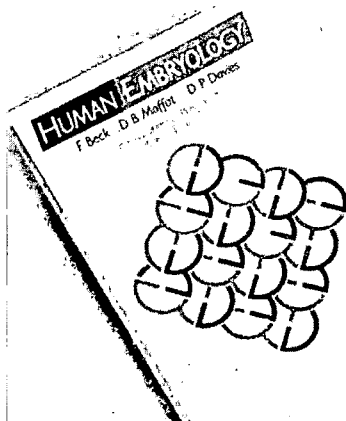
Current Trends in Reproductive Endocrinology, Infertility and Contraception, August 9-15, 1986, Waimea Beach Hotel, in Maui, Hawaii. For information contact: Beverly J. Johnson, Program Specialist, University of Southern California, Postgraduate Medical Education, 2025 Zonal Ave., Los Angeles, CA 90033. Tel.: (213) 224-7051.

1987 American Society of Andrology, March 6-9, 1987, Denver, Colorado. Abstracts due October 10, 1986. For further information, contact: W. D. Odell, M.D., Ph.D., Department of Medicine, University of Utah College of Medicine, 50 North Medical Dr., Salt Lake City, UT 84132. Tel.: (801) 581-7606.

Call for papers for the Third International Congress on Pre and Perinatal Psychology, San Francisco, California, July 10-12, 1987. Deadline for abstracts is November 15, 1986. For information contact: Mary L. Davenport, M.D., 5309 College Ave., Oakland, CA 94618. Tel.: (415) 420-1000.

Perinatal Medicine: 1986—Back to Basics, October 16-17, 1986, Northwestern University Medical School, Chicago, Illinois. For information contact: Paula Puntenney, Director, Alumni Center for Continuing Education, Northwestern University Medical School, 301 E. Chicago Ave., Chicago, IL 60611. Tel.: (312) 908-8533.

Gynecologic Urology Society, Seventh Annual Clinical Meeting and Post Graduate Course, October 1-4, 1986, Le Chateau Champlain-Montreal, Canada. For further information contact: David A. Richardson, M.D., Secretary/Treasurer, c/o Grace Hospital, Department of Obstetrics and Gynecology, 18700 Meyers Road, Detroit, MI 48235. Tel.: (313) 966-3246.



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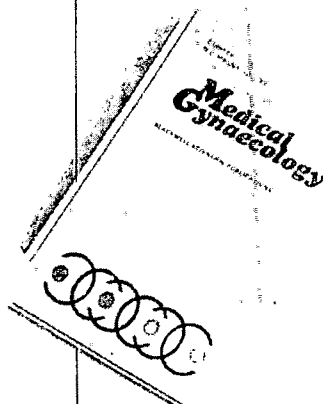
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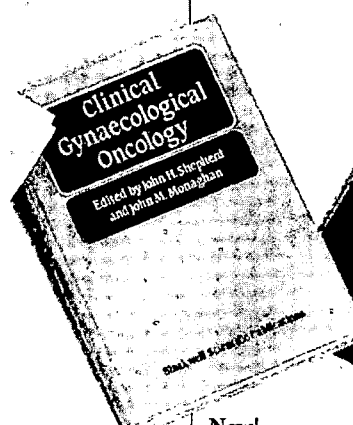
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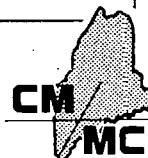
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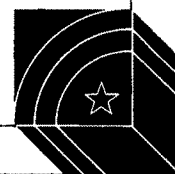
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Section II: SERIOUS OBSTETRIC PROBLEMS
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Section III: SPECIFIC PROBLEMS OF THE
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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogens. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 0.05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking the drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS.** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. The overall excess mortality due to pulmonary embolism or stroke is of the order of 10 to 35 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/tokemia), the higher the risk of developing myocardial infarction; regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor in determining the importance of these relative risks; however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism and other vascular diseases. Their analysis also suggested, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 ages 15-34, 5-100,000 ages 35-44, 33-100,000 ages 45-49, 140-100,000 ages 50-54, being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double. For "light" smokers (less than 15 cigarettes a day), about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/tokemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women aged 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. **CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogen. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. **HEPATIC TUMORS:** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral

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contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4- to 14-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy as a method of treating threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuing the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. **GALLBLADDER DISEASE:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBOHYDRATE AND LIPID METABOLIC EFFECTS:** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and low-density lipoproteins has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE:** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives, and evaluation of the cause. 10. **BLEEDING/IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postulate abortion possibly prolonged, may also occur in women with previous irregularities. 11. **ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **EARLY FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogenic preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution and only with careful monitoring in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive use may have adverse effects on normal triglyceride metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention; b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin; c. increased norepinephrine-induced platelet aggregability; d. increased thyroid-binding globulin (TBG) leading to increased circulating thyroid hormone, as measured by protein bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; e. Decreased pregnanediol excretion; f. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS AND WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS). Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions (e.g., retinal thrombosis and optic neuritis). The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine, increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

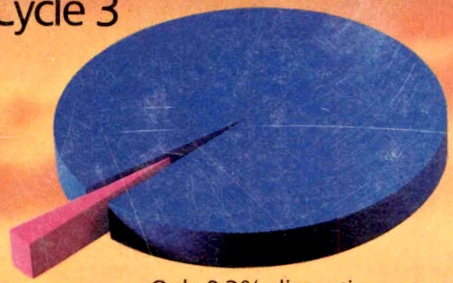


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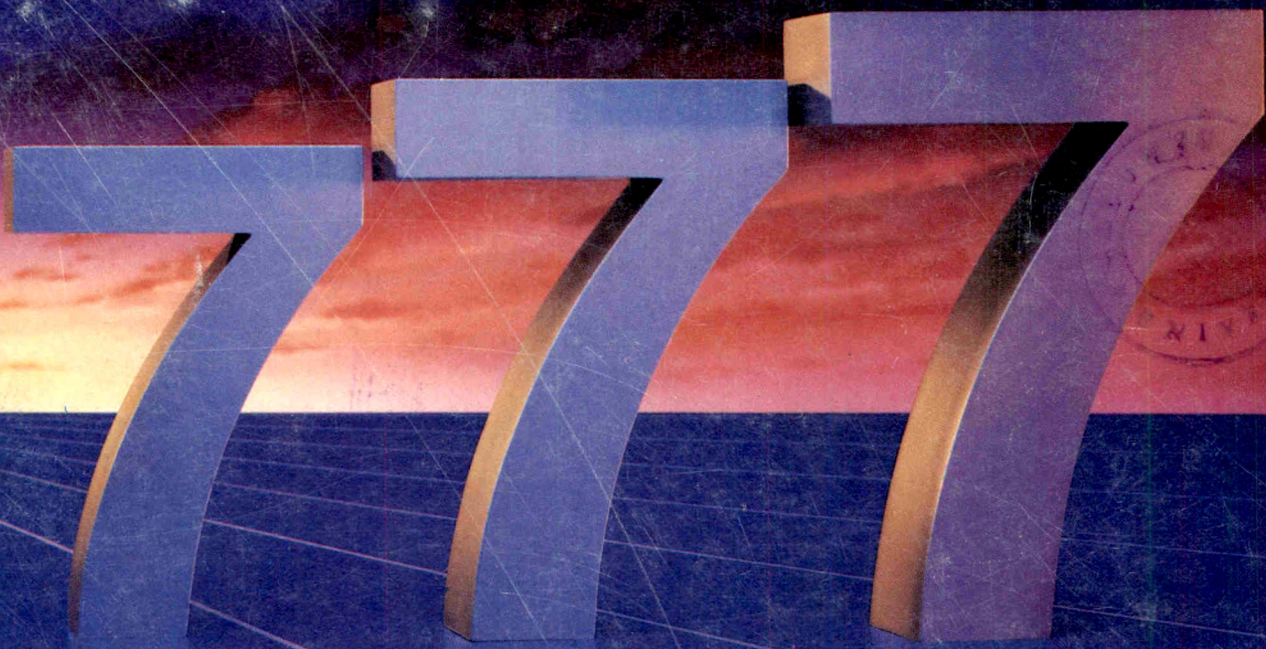
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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see complete Prescribing Information, a summary of which appears on the preceding page.

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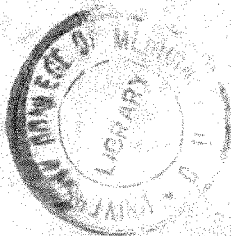
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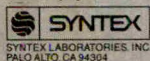
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(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Data available from Syntex Laboratories, Inc.

**Based on a nationwide, independently conducted survey of prospective oral contraceptive patients. Data available from Syntex Laboratories, Inc.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.



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THE NATURE OF TRI-NORINYL[®]

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL[®] 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 10 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL[®] 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 10 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications: Prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM:** Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction; b. known or suspected carcinoma of the breast; c. known or suspected estrogen dependent neoplasia; d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism, CEREbrovascular DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI): Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the risk of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclinical toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that non-smoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to develop MI as nonusers. In the U.S., death rates for MI are smokers have a 5-fold increased risk of fatal infarction compared to non-smoking users, and a 10-12 fold increased risk compared to non-smoking non-users. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S., so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified, other synergistic causes may exist. **RISK OF DOSE:** Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 10 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. **PERSISTENCE OF RISK:** Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall excess death rate for OC users was estimated to be 20/100,000 (ages 15-34: 5/100,000, ages 35-44: 33/100,000, ages 45-49: 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates, ages 15-34: 16/100 for nonusers; 45-49: 16/100 for smokers; 45-49: 1/2500 for nonusers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 years of age. For women 15-39, only smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half. The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of preclinical toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonusers	D	C,D	C
(no predisposing conditions)			
Nonusers	D	C,D	C
(other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk.
B—Use associated with high risk.
C—Use associated with moderate risk.
D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. **Ocular Lesions:** Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. **Carcinoma:** Long-term continuous administration of natural or synthetic estrogen to certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, now currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (31-139 times) associating endometrial carcinoma with long-term use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistically significant association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer to OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term use. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increased risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancer. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence of human cancer associated with OC use, nevertheless, associated with OCs. Close clinical surveillance of all OC users is nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. **Liver Tumors:** Sudden severe abdominal pain, nausea, and/or vomiting, and/or jaundice or hemoconcentration, have been reported in association with the hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, being much greater with 10 years or more of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3.4-100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most masses, when discovered, were acute subcapsular hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasms. 5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** Use of female sex hormones, estrogenic, and progestational agents—during early pregnancy may seriously damage the offspring. Fetal effects among offspring of prior OC users (nonsteroidal estrogens, have an increased risk of developing in late life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be managed with particular care. A high percentage of women exposed to diethylstilbestrol (DES) have epithelial cancer of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 182/1000 births, compared to 78/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestagens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortions from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortions of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient the potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. **Gall Bladder**

Disease: Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. **Carbohydrate and Lipid Metabolic Effects:** Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users. But its clinical significance is unknown. 8. **Elevated Blood Pressure:** An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors (hypertension, diabetes or stroke). High blood pressure may or may not persist after OC discontinuation. 9. **Headache:** Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, missed menses, or other menstrual changes may occur. In breaking through bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea and galactorrhea was present. 11. **Infertility:** There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after discontinuation. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is enhanced by OCs. 12. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. **Breast Feeding:** OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions:** GENERAL. 1. Take a complete medical and family history before starting OCs. 2. Perform a physical examination, including pelvic exams, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression occurs. Advise the patient of the possibility of becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is OC related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution to such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxamine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. increased sulfobromophthalen retention; b. increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin; c. increased norepinephrine-induced platelet aggregation; d. increased thyroxine binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin-bound is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered; d. decreased pregnandiol excretion; e. reduced response to metoprolol test; f. increased phospholipids and triglycerides; g. temporarily decreased glucose tolerance; h. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. **DRUG INTERACTIONS:** OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, antacids, anticholinergics, tranquilizers, antidiuretics, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. **CARCINOGENESIS:** See Warnings. **PREGNANCY:** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS:** See Warnings. **Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings). Thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebrothrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, stroke-like syndromes, cataracts, changes in libido, cholestatic jaundice, migraine, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastroenteric symptoms (nausea, vomiting, bloating, headache, changes in appetite, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical reaction, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chloasma. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, cholestatic jaundice, migraine, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient:** See Patient Package Insert.

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WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. 2. Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. 3. Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. 4. Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. 5. Usage in pregnancy is not recommended (see Warning box). 6. Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influ-

enced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginam, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T₃ uptake values).

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TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

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American Journal of Obstetrics and Gynecology

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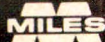
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INDICATIONS: Mycelex-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis, *Trichomonas* and *Gardnerella (Haemophilus) vaginalis*, should be ruled out by appropriate laboratory methods.

CONTRAINDICATIONS: Mycelex-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

WARNINGS: None.

PRECAUTIONS: If there is a lack of response to Mycelex-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

CARCINOGENESIS: No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

USAGE IN PREGNANCY: PREGNANCY CATEGORY B: The disposition of ¹⁴C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

ADVERSE REACTIONS: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

OVERDOSAGE: No data available.

DRUG ABUSE AND DEPENDENCE: Drug abuse and dependence with Mycelex-G 500 mg Vaginal Tablets has not been reported.

DOSEAGE AND ADMINISTRATION: The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

HOW SUPPLIED: Mycelex-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with MILES on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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The first edition of this book was described as "outstandingly successful, there can be few clearer or more comprehensive accounts of the physiological basis of modern obstetrics" *British Medical Journal*. 823 pages 1984 \$70.

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Why risk significant *Bacteroides fragilis*

**Cefotan has gaps* in its coverage
of these *B. fragilis* group** organisms.**

MEFOXIN does not.

Clinical indications for intraabdominal infections†		
	Cefotan^{††} (cefotetan disodium)	Mefoxin[®] (Cefoxitin Sodium MSD)
<i>Bacteroides</i> species		
including <i>B. fragilis</i> ‡		
<i>B. distasonis</i>		
<i>B. ovatus</i>		
<i>B. thetaiotaomicron</i>		
<i>B. vulgatus</i> ‡		

†MEFOXIN is indicated for the treatment of intraabdominal infections, including peritonitis and intraabdominal abscess, caused by *Escherichia coli*, *Klebsiella* species, *Bacteroides* species including the *Bacteroides fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*), and *Clostridium* species.

††Cefotan is a registered trademark of ICI Americas Inc.
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**These organisms, formerly considered subspecies of *B. fragilis*, have been given species status; however, because of similarities in their clinical significance and laboratory characteristics, they are often considered together as the "*B. fragilis* group."

‡The prescribing information for Cefotan lists no specific indication for infections caused by this organism.¹

gaps* in coverage?

All five of these *Bacteroides* are proven pathogens.

The evidence is conclusive.

fact: *B. fragilis* and *B. thetaiotaomicron* are the species of greatest clinical significance and are present in most intraabdominal infections.

fact: The prescribing information for Cefotan specifically states that most strains of *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron* are resistant.¹

fact: The absence of susceptibility of these *Bacteroides fragilis* species to Cefotan clearly shows that Cefotan does not have the same anaerobic spectrum as MEFOXIN.

Conclusion: Cefotan is not equal to MEFOXIN in activity^{##} against the *B. fragilis* group.

the
facts *still*
favor
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MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use.

*i.e., *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron*

^{##} *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Prescribing information for Cefotan (Stuart), March 1986.

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For a Brief Summary of
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Mefoxin[®] _{TM} (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group, and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTAXIME, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOTAXIME OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia: a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffe reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINTEST II reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk; Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently.

Local Reactions—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. **Cardiovascular**—Hypotension. **Gastrointestinal**—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. **Blood**—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

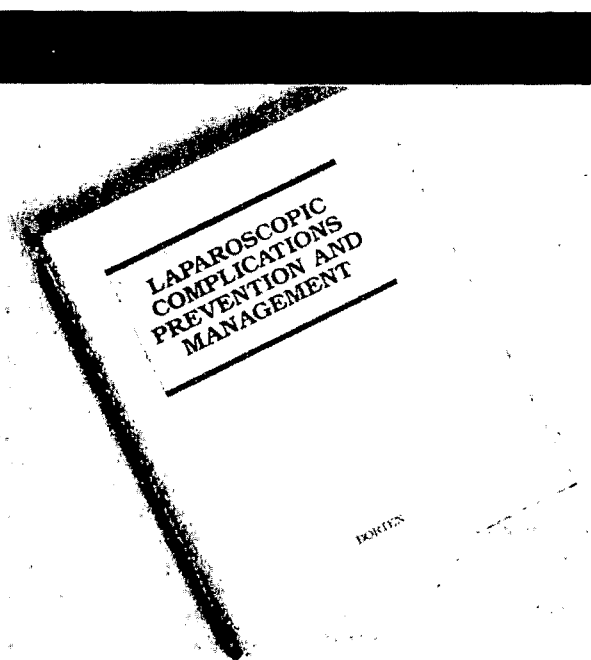
Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

S. B. fragilis, *B. distans*, *B. ovalis*, *B. thetaiotaomicron*, *B. vulgatus*
[†] Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

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CLINICAL SECTION

Clinical Opinion

Acquired immunodeficiency syndrome: Epidemiology and significance for the obstetrician and gynecologist

David J. Weber, M.D., M.P.H., Robert R. Redfield, M.D., and Stanley M. Lemon, M.D.

Chapel Hill, North Carolina, and Washington, D. C.

A retrovirus, variously named human T-lymphotropic virus type III or lymphadenopathy-associated virus, is now recognized as the etiologic agent of the acquired immunodeficiency syndrome. As of January, 1986, more than 16,000 cases of acquired immunodeficiency syndrome have been reported, and it is estimated that ≥ 1 million Americans are now infected with human T-cell lymphotropic virus/lymphadenopathy-associated virus. Most cases of overt acquired immunodeficiency syndrome still fall within well-defined "high-risk groups," that is, homosexual or bisexual men, intravenous drug users, recipients of transfused blood or blood components, and persons with hemophilia. Also at risk are heterosexual partners of persons who have overt acquired immunodeficiency syndrome or who are at risk for acquired immunodeficiency syndrome, as are children born to mothers who either have acquired immunodeficiency syndrome or are members of a "high-risk group." Transmission of the virus may occur via sexual contact, via parenteral exposure to contaminated blood products, or from an infected mother to her child. Of special concern to the practicing obstetrician are recent reports suggesting bidirectional heterosexual transmission, transmission via artificial insemination, and perinatal transmission. In addition, the isolation of human T-cell lymphotropic virus/lymphadenopathy-associated virus from human breast milk raises concerns regarding potential transmission by this route. (AM J OBSTET GYNECOL 1986;155:235-40.)

Key words: Acquired immunodeficiency syndrome, AIDS, human T-cell lymphotropic virus type III

The first clinical descriptions of the acquired immunodeficiency syndrome (AIDS) were reported in 1981, when groups in New York and San Francisco described the occurrence of *Pneumocystis carinii* pneumonia or Kaposi's sarcoma in previously healthy homosexual men. The case definition of AIDS given below was published by the Centers for Disease Control in September, 1982, and has since been periodically updated. The definition includes persons with unexplained opportunistic infections and certain malignancies that are indicative of an underlying acquired immunodeficiency. The etiologic agent of AIDS was first isolated in 1983, independently by French and American investigators, and named lymphadenopathy-

associated virus (LAV) or human T-cell lymphotropic virus type III (HTLV-III).^{*} A symposium detailing the clinical manifestations, basic virology, and epidemiology has recently been published.¹

For surveillance purposes in adults, AIDS is defined as the occurrence of biopsy-proved Kaposi's sarcoma and/or biopsy- or culture-proved infection at least moderately predictive of cellular immune deficiency. In the original definition patients who either had received immunosuppressive therapy before the onset of illness or had preexisting illnesses associated with immunosuppression, such as congenital immunodeficiency or lymphoreticular malignancy, were excluded. Also excluded were persons with Kaposi's sarcoma who were more than 60 years of age.

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**The designation human immunodeficiency virus (HIV) has recently been proposed by a subcommittee of the International Committee for the Taxonomy of Viruses as the appropriate name for the retrovirus that has been implicated as the causative agent of AIDS.*

Infections considered at least moderately predictive of underlying cellular immune deficiency are as follows*:

A. Protozoal and helminthic infections

1. Cryptosporidiosis, intestinal, causing diarrhea for >1 month (on histologic examination or stool microscopy)
2. Pneumocystis carinii pneumonia (on histologic examination or microscopy)
3. Strongyloides, causing pneumonia, central nervous system infection, or disseminated infection (on histologic examination)
4. Toxoplasmosis, causing pneumonia or central nervous system infection (on histologic examination or microscopy)

B. Fungal infections

1. Aspergillosis, causing central nervous system or disseminated infection (on culture or histologic examination)
2. Candidiasis, causing esophagitis (on histologic examination, microscopy of a "wet preparation" from the esophagus, or endoscopic findings of white plaques on an erythematous base)
3. Cryptococcus, causing pulmonary, central nervous system, or disseminated infection (on culture, antigen detection, histologic examination, or India ink preparation of cerebrospinal fluid)

C. Bacterial infections

1. "Atypical" mycobacteriosis (species other than *Mycobacterium tuberculosis* or *Mycobacterium leprae*), causing disseminated infection (on culture)

D. Viral infection

1. Cytomegalovirus, causing pulmonary, gastrointestinal tract, or central nervous system infection (on histologic examination)
2. Herpes simplex virus, causing chronic mucocutaneous infection with ulcers persisting >1 month or pulmonary, gastrointestinal tract, or disseminated infection (on culture, histologic examination, or cytologic testing)
3. Progressive multifocal leukoencephalopathy (presumed to be caused by papovavirus) (on histologic examination)

The revised definition is as follows:

- A. In the absence of the opportunistic diseases required by the above case definition, any of the following diseases will be considered indicative of AIDS in the patient who has a positive serologic or virologic test for HTLV-III/LAV infection

1. Histoplasmosis, disseminated (not confined to lungs or lymph nodes) (on culture, histologic examination, or antigen detection)

2. Isosporiasis, intestinal, causing diarrhea for >1 month (on histologic examination or microscopy)

3. Candidiasis, bronchial or pulmonary (on microscopy or by presence of characteristic white plaques grossly on the bronchial mucosa—not by culture alone)

4. Non-Hodgkin's lymphoma of high-grade pathogenic type (diffuse, undifferentiated) and of B-cell or unknown immunologic phenotype, diagnosed by biopsy

5. Kaposi's sarcoma, in patient >60 years of age (on histologic examination)

- B. Patients who have a lymphoreticular malignancy diagnosed >3 months after the diagnosis of an opportunistic disease used as a marker for AIDS will no longer be excluded as AIDS cases

- C. Patients will be excluded as AIDS cases if they have a negative result on testing for serum antibody to HTLV-III/LAV, have no other type of HTLV-III/LAV test with a positive result, and do not have a low number of T-helper lymphocytes or a low ratio of T-helper to T-suppressor lymphocytes. In the absence of test results, patients satisfying all other criteria in the definition will continue to be included

Investigation of the epidemiology of HTLV-III/LAV infection was initially based on case detection with the Centers for Disease Control case definition. However, isolation of the etiologic agent has more recently allowed seroepidemiologic surveys based on detection of anti-HTLV III/LAV antibodies with the use of enzyme-linked immunosorbent assays and Western blot techniques. As of January 13, 1986, 16,458 cases of AIDS had been reported to the Centers for Disease Control.² The number of cases reported to the Centers for Disease Control continues to increase, although not exponentially. It is estimated that the number of reported cases of AIDS will approximately double between January, 1986, and January, 1987. Among the adult cases recognized, 65% have occurred in homosexual or bisexual men who are not known intravenous drug users, 17% in heterosexual intravenous drug users, 8% in homosexual or bisexual intravenous drug users, 2% in persons receiving transfused blood or blood components, and 1% in patients with hemophilia or other coagulation disorders. In addition, 1% have been considered to have acquired infection through heterosexual contact with a patient known to have AIDS or a member of a high-risk group, while 6% could not be definitely placed in any of these categories. Recent reports indicate that transfusion-related cases currently constitute a slightly increased proportion of cases. Overall 93% of adult cases have occurred in men but an increased proportion of newly diagnosed cases are in women. The proportion of cases not belonging to a known risk group continues to remain constant but may be altered with prospective active surveillance. In many

*Modified from Jaffe HW, Bregman DJ, Selik RM. Acquired immune deficiency syndrome in the United States: the first 1,000 cases. *J Infect Dis* 1985;148:340; and from Centers for Disease Control. Revision of the case definition of acquired immune deficiency syndrome for national reporting—United States. *MMWR* 1985;34:373-5.

of these cases, the data base is incomplete and relevant risk factors may have been missed.

The Centers for Disease Control recently published a provisional case definition of pediatric AIDS. For the limited purposes of epidemiologic surveillance, Centers for Disease Control defines a case of pediatric AIDS as a child who has had: (1) a reliably diagnosed disease at least moderately indicative of underlying immunodeficiency and (2) no known cause of underlying cellular immunodeficiency or any other reduced resistance reported to be associated with that disease.

The diseases accepted as sufficiently indicative of underlying cellular immunodeficiency are the same as those used in defining AIDS in adults. In the absence of these opportunistic diseases, a histologically confirmed diagnosis of chronic lymphoid interstitial pneumonitis will be considered indicative of AIDS unless test(s) for HTLV-III/LAV are negative. Congenital infections, for instance, toxoplasmosis or herpes simplex virus infection in the first month after birth or cytomegalovirus infection in the first 6 months after birth, must be excluded.

Specific conditions that must be excluded in a child are: (1) primary immunodeficiency diseases, such as severe combined immunodeficiency, DiGeorge syndrome, Wiskott-Aldrich syndrome, ataxia-telangiectasia, graft versus host disease, neutropenia, neutrophil function abnormality, agammaglobulinemia, or hypogammaglobulinemia with raised IgM; (2) secondary immunodeficiency associated with immunosuppressive therapy, lymphoreticular malignancy, or starvation.*

Congenital immunodeficiency disorders must be ruled out. Approximately 1.4% of AIDS cases have occurred in individuals under the age of 13.² Seventy-five percent of these pediatric cases occurred in children whose parents had AIDS or were at increased risk for AIDS. In most instances, epidemiologic data suggested transmission from infected mother to child. Among the remaining cases, 5% occurred in children with hemophilia or other coagulation disorders, and 14% were in children who had received blood or blood products, while 6% could not be placed in any of these categories. Reports of pediatric cases suggest an incubation period of <1 year in most cases of perinatal transmission. Estimates of incidence rates suggest that children receiving blood products may have a higher risk of acquiring transfusion-related AIDS than adults.

AIDS is now recognized as the most severe manifestation of a spectrum of HTLV-III/LAV-associated diseases.³ The causative agent of AIDS is a retrovirus, a viral agent containing ribonucleic acid that replicates by means of a deoxyribonucleic acid intermediate that

may be integrated into host cell deoxyribonucleic acid. HTLV-III/LAV infection may lead to asymptomatic carriage, AIDS, or a spectrum of lymphadenopathy-associated syndromes grouped as AIDS-related complex (ARC). These syndromes are characterized by persistent lymphadenopathy for >3 to 6 months in the absence of an alternative cause. Epidemiologic studies suggest that ARC is at least sevenfold more common than AIDS and that exposure to HTLV-III/LAV virus has occurred in ≥ 1 million Americans. Serologic surveys have revealed anti-HTLV-III/LAV antibodies in 90% to 100% of patients with AIDS, 90% to 100% of patients with "lesser AIDS," 70% to 90% of patients with lymphadenopathy syndromes, 50% to 70% of homosexual men (high-risk area), 10% to 50% of homosexual men (low- to intermediate-risk area), 50% to 70% of intravenous drug users (high-risk area), 1% to 30% of intravenous drug users (low- to intermediate-risk area), 70% to 90% of hemophiliacs (factor VIII concentrate therapy), 10% to 40% of hemophiliacs (other therapy), and <0.25% of unselected blood donors.⁴ Rates of seropositivity are likely to vary depending on the location and nature of the population being sampled. HTLV-III/LAV virus can be isolated from most seropositive patients. Thus, unlike in many viral infections, the presence of antibody is not protective but instead is indicative of current, active infection. Many, if not most, seropositive persons are probably capable of transmitting HTLV-III/LAV infection.

The frequency with which HTLV-III/LAV-infected individuals will develop a chronic asymptomatic carrier state, immunologic abnormalities, lymphadenopathy, ARC, or AIDS has not been well characterized. While only crude estimates are available, it appears that 2% to 9% of seropositive persons may develop AIDS each year.⁴ As described above, the development of the lymphadenopathy syndrome appears to be more common than AIDS among seropositive persons. More than 25% of cases of lymphadenopathy syndromes may progress to AIDS.

HTLV-III/LAV has been isolated from peripheral blood, bone marrow, lymph nodes, brain tissue, cerebrospinal fluid, semen, saliva, tears, human breast milk, and cervical secretions. The virus is most frequently transmitted via human sexual contact and to a lesser extent via sharing of contaminated needles among intravenous drug users or administration of blood or blood products. Maternal-child transmission and transmission during artificial insemination are also possible. Heterosexual transmission does occur, with those persons at greatest risk of acquiring infection by this route being the steady partners of persons who either have overt AIDS or are seropositive and in a high-risk group. Bidirectional sexual transmission (that is, male-to-female and female-to-male) of HTLV-III/LAV appears

*Case definition modified from Centers for Disease Control. Education and foster care of children infected with human T-lymphotropic virus type III/lymphadenopathy associated virus. MMWR 1985;34:518.

likely given current data. While male-to-female transmission may occur with a higher frequency than female-to-male transmission, the relative efficiency of transmission in each direction is still unknown.

Blood products reported to transmit HTLV-III/LAV include red blood cells, platelets, plasma, whole blood, clotting factor concentrates, and probably cryoprecipitate. Heat treatment of clotting factor concentrates appears to very much lessen the probability that this vehicle may serve as a source of transmission. Screening of blood for anti-HTLV-III/LAV antibodies should reduce the likelihood of transfusion-related AIDS but will not totally eliminate blood as a mode of transmission since some HTLV-III/LAV-infected persons are seronegative (estimated at 5% to 15%). Also many transfusion-acquired cases of AIDS will continue to be recognized as the incubation period for transfusion-related AIDS may be many years. Epidemiologic studies have demonstrated no increased incidence of AIDS in recipients of the hepatitis B plasma vaccine. Further, *in vitro* studies indicate that several of the inactivation steps used in vaccine production effectively reduce HTLV-III/LAV to undetectable levels. Likewise immunoglobulins have not been linked to transmission of HTLV-III/LAV.

Transmission of HTLV-III/LAV from mother to child may occur via three possible routes: transplacental, intrapartum exposure to infected maternal blood, or postnatal exposure to breast milk or other close mother-to-infant contact. Case reports have suggested that all three modes of infection do in fact occur. Jovaisas et al.⁵ demonstrated HTLV-III/LAV antigens in the organs of a 20-week abortus. Case reports have documented AIDS in children born to high-risk mothers despite delivery by cesarean section^{6,7} or removal at birth to foster families.⁸ The recent isolation of HTLV-III/LAV from breast milk suggests that this could be a vehicle for viral transmission,⁸ although it remains speculative whether viral infection can be acquired by the oral route. Ziegler et al.⁹ have reported a case where a woman received a postdelivery transfusion from a person who later developed AIDS; both mother and child are seropositive 17 months after delivery, suggesting breast-feeding as a mode of maternal-child transmission.

The magnitude of the risk of maternal-child transmission of HTLV-III/LAV is still unclear. In a study of 20 infants born to mothers who had previously been delivered of an infant with AIDS, 13 infants (65%) underwent seroconversion or developed clinical evidence of HTLV-III/LAV infection within 1 year.^{10,11} A retrospective study of nine children <5 years of age whose mothers were later diagnosed with AIDS revealed two children (22%) to be seropositive.¹² A 1985 report from Italy revealed that 11 of 12 children born

to HTLV-III/LAV-positive mothers were seropositive (91%); five had AIDS (41%) and two had ARC (16%).¹³

The epidemic of HTLV-III/LAV infection poses many problems for the obstetrician that deserve comment. HTLV-III/LAV has been isolated from semen and cervical secretions; sexual transmission is a major mode of transmission. HTLV-III/LAV-infected men should be encouraged to use condoms as a means of preventing transmission to their sexual partners, although specific proof of efficacy is lacking. Recently artificial insemination has been reported to have resulted in transmission of HTLV-III/LAV.¹⁴ For this reason all sperm donors should be screened by enzyme-linked immunosorbent assay for HTLV-III/LAV antibodies and individuals with repeatedly positive or Western blot-positive results excluded as donors. Since newly infected persons may be seronegative but viremic for a period of 2 to 4 months, it may be prudent to store sperm and use only donors who continue to test seronegative 3 to 6 months after sperm donation. Most important of all, members of high-risk groups should be excluded from sperm and organ donation even if HTLV-III/LAV negative. Based on reports of HTLV-III/LAV isolation from human breast milk and the possibility of postnatal transmission of virus, all centers using human breast milk should exclude donors belonging to high-risk groups and should screen all other donors for HTLV-III/LAV antibodies.

We agree with the recommendations of the Centers for Disease Control that all high-risk women should be offered screening for HTLV-III/LAV antibodies and appropriately counseled if results are positive.¹⁵ Such counseling should include a discussion of the possibility that offspring will develop AIDS, progression of HTLV-III/LAV infection to AIDS in pregnant women, and the possibility of viral transmission to sexual partners. Recent Centers for Disease Control guidelines suggest that screening should be offered to pregnant women in the following groups: (1) individuals with clinical evidence of HTLV-III/LAV infection; (2) intravenous drug abusers; (3) individuals born in countries where heterosexual transmission is thought to play a major role in the epidemiology of the virus; (4) women engaged in prostitution; (5) women who are or have been sexual partners of bisexual men, men with hemophilia, men with evidence of HTLV-III/LAV, or men who were born in countries where heterosexual transmission is thought to play a major role.

Epidemiologic investigations have revealed that HTLV-III/LAV may be transmitted from asymptomatic mothers to their offspring and that such children are at risk for developing AIDS. Similarly, detection of a case of pediatric AIDS should lead to investigation of the mother as many mothers will be infected, although asymptomatic. In such cases there is a significant risk

that future pregnancies will lead to infected children. However, the exact frequency with which infected mothers, whether asymptomatic or symptomatic, will transmit HTLV-III/LAV to their infants and the subsequent risk that the child will develop ARC or AIDS is currently unknown. In addition, there is some tentative evidence to suggest the the pregnant state may favor the progression of HTLV-III/LAV infection to AIDS or ARC. This may not be surprising given the alterations in immune function known to occur in pregnancy.

Whether all pregnant women should routinely be tested for HTLV-III/LAV infection will depend on future prevalence rates in the general population and ethical and legal issues. Although the current enzyme-linked immunosorbent assay is sensitive, it does not have good predictive value when used to screen the general population. Approximately two of every three repeatedly positive tests in unselected blood donors are considered false positive, based on Western blot and virus isolation confirmation.

HTLV-III/LAV appears to pose only a small nosocomial risk. Prevention of possible nosocomial spread requires adherence to infection control guidelines.¹⁶ HTLV-III/LAV appears susceptible to a variety of commonly used disinfectants. The following agents have been reported to reduce five logs of virus to undetectable levels within 10 minutes: 0.3% hydrogen peroxide, 50% ethyl alcohol, 35% isopropyl alcohol, 0.5% paraformaldehyde, 0.5% Lysol, and 0.1% household bleach. All specula and diaphragm fitting devices should be sterilized or high-level disinfected after patient use. An ambu-bag, oral airway, and goggles should be placed in the rooms of all HTLV-III/LAV patients to eliminate the need for mouth-to-mouth respiration in the event of cardiopulmonary arrest.

Transmission of HTLV-III/LAV by casual contact has not been reported. Parenteral exposure to HTLV-III/LAV-containing blood has been reported among hospital personnel, most commonly as the result of needle-stick injuries. However, intensive follow-up has thus far revealed that only a small number of hospital staff (<1%) may have acquired HTLV-III/LAV infection following such exposure. Few reports carefully document seroconversion and rule out the possibility of sexual transmission of the virus. Nevertheless, caution should be used when dealing with all blood and secretory products.

Given the extent of the current epidemic and the fact that it involves individuals with a peak age between 20 and 49, all practicing obstetricians and gynecologists should expect that they will encounter, in clinical practice, patients who are asymptomatically infected with HTLV-III/LAV or even patients with ARC or AIDS. Obstetricians and gynecologists must be familiar with

the HTLV-III/LAV virus and its varied clinical manifestations. Difficult decisions now confronting obstetricians faced with women who are infected with HTLV-III may be in part resolved by future epidemiologic studies that should better define the frequency of maternal-child transmission, risks to the fetus and newborn infant, and consequences to the pregnant women.

Addendum

Since this manuscript was accepted the Centers for Disease Control has published a new classification scheme for HTLV-III/LAV infections and additional recommendations designed to prevent or minimize HTLV-III/LAV transmission.¹⁷⁻¹⁹

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Critical issues in prenatal maternal serum α -fetoprotein screening for genetic anomalies

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Prenatal screening, in this Clinical Opinion article, is defined as a population-based search for subgroups of pregnancy that by virtue of their maternal serum α -fetoprotein levels may be at increased risk for genetic anomalies. The identification of subgroups at risk for (1) neural tube defects, (2) ventral wall defects, and (3) chromosomal trisomies presents clinicians with unprecedented information and issues, including the biomedical basis for prenatal screening, the distinction between screening and diagnosis, and the decision-making function of the patient. The interest and, perhaps, ambivalence of the clinical community to prenatal screening are understandable. Obstetricians ordering prenatal screens are challenged by medical data associated with epidemiology, pathology, and genetics, including: (1) prevalence of disease, (2) interpretive biochemical reporting, and (3) the assessment and communication of patient-specific risks. Obstetricians ordering screens will be called on to provide information generally used by other specialists at other stages of care. What information, how it is biomedically determined, its form for communication to patients, and pitfalls in the process are discussed. (*AM J OBSTET GYNECOL* 1986;155:240-6.)

Key words: Screening, AFP, open neural tube defect, risk reporting, low MSAFP

Effective communications to patients concerning the risk of carrying a fetus with an open neural tube defect (NTD) should be based on knowledge of the patterns of incidence of these congenital disorders in subgroups of pregnancies. Such knowledge fosters the assessment of risk by both physician and patient and leads to informed decision-making.

In certain pregnancy subgroups the risk of occurrence or recurrence of NTD is well established.¹ For example: (1) families with offspring affected by NTD demonstrate recurrence rates of approximately 2% and (2) pregnancies with amniocentesis performed for reasons other than a history of NTD (the majority fall within the category of advanced maternal age) have

offspring with NTD at a rate of approximately 0.003% (1/295).

Since the mid-1970s, documentation supporting the diagnostic usefulness of amniotic fluid α -fetoprotein (AFP) and more recently acetylcholinesterase for detection of open NTD has been available and obstetricians are aware of the risk in the foregoing pregnancy subgroups. By use of amniotic fluid AFP evaluations in an established clinical protocol, families at increased risk are receiving, through prenatal diagnosis, greatly needed reassurances resulting in the birth of many normal, healthy children. It should be emphasized that these pregnancies (at increased risk) can receive accurate diagnostic testing and information.

However, since greater than 95% of newborn infants with NTD occur in families with unremarkable histories, the need for a viable screening protocol in the general pregnant population (where the incidence of

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open NTD may be $\sim 1/500$) is clear. Sizable pilot screening programs using maternal serum AFP to identify pregnancies at increased risk have demonstrated that in carefully controlled screening protocols, pregnancies with elevated maternal serum AFP during the second trimester may face risks of NTD of 5% or higher.² Such programs have established that prenatal maternal serum AFP screening can identify a subgroup of pregnancies with the highest known risk of NTD. In spite of this increased risk, however, it should be noted that the least likely cause of an elevated level of maternal serum AFP is the presence of a fetus with NTD. For example, gravid women may demonstrate significant maternal serum AFP elevations (1) in normal multiple gestations, (2) in cases of underestimated gestational age (maternal serum AFP normally increases with each week of pregnancy during the second trimester), (3) as a result of specific placental changes causing fetomaternal transfusion, and (4) in association with normal fetuses. These factors underscore the screening rather than diagnostic nature of maternal serum AFP evaluations in desired pregnancies.

Since prenatal screening of pregnancy populations for the detection of genetic disorders is unprecedented and because the total United States experience with maternal serum AFP screening has been limited (perhaps less than 5% of pregnancies have been screened in any given year) the tendency in the biomedical laboratory community has been to approach prenatal maternal serum AFP screening as though it were a diagnostic process. Normative data bases are borrowed so that some laboratories operate before developing their own (leading to problems with interpretation). Proficiency test programs for AFP are established along traditional lines (quantitative comparability) and reports of maternal serum AFP screening are given in conventional quantitative units (implying normality or abnormality). These approaches are misguided and unreasonable because prenatal maternal serum AFP screening should not follow a diagnostic but rather a screening protocol.

Screening should result in the identification of a subgroup in which at least two criteria are met: (1) any gravid woman screened into the subgroup should be at sufficient risk to warrant offering further diagnostic testing (which in itself may carry risk) and (2) fetuses with open NTD in the screening population should be found within the identified subgroup.

We will illustrate that in order to meet these criteria, (1) independently developed normative data bases are essential in each laboratory, (2) proficiency testing should assess "real-world" maternal serum AFP screening, (3) reporting in conventional quantitative units is inappropriate, and (4) patient-specific risk communication is clinically preferable.

Normative data

Fundamental to laboratory interpretive accuracy and clinical value is the independent development of a normative data base. There are a number of known and suspected factors that cause interlaboratory variation in quantitation and interpretation. The utilization of a normative data base established by any other laboratory or other source lacks scientific validity. Information from one AFP quality assessment scheme indicates³:

In most cases the problems with interpretation arise from inaccurate reference data (medians). The QC program has worked with several of these laboratories to attempt to rectify some of the problems but has been relatively unsuccessful in convincing one of the laboratories that a problem exists. There seems to be a disturbing tendency to rationalize away the poor performance by resorting to speculation that populations are different or that the group consensus is not a reasonable standard for judging performance.

Professional standards demand that valid normative data be established independently by each laboratory. This process will account for the uniqueness of internal testing conditions, which include, but are not limited to, the specific test reagents used, the type of testing equipment (automatic versus manual), experience and proficiency of technicians, quality of water, efficiency of counting equipment, mode of data reduction, and quality of samples being analyzed.

Nevertheless, laboratories do and may continue to initiate maternal serum AFP screening with the use of manufacturer's package insert data and experience problems with interpretation. In response, some health authorities have enacted regulations to foster the development of independent data bases. For example, one state department of health requires that maternal serum AFP laboratory directors "demonstrate experience in the measurement of at least 3,000 human serum and/or amniotic fluid determinations."⁴ This specimen-volume regulation is intended to require laboratories to develop sufficient experience to detect at least three positive cases (spina bifida and/or anencephaly) and conduct subsequent follow-up procedures. Additionally, the called-for experience is intended to allow for the accumulation of sufficient samples to obtain adequate gestational week AFP cutoff values (a minimum of 100 samples for each gestational week is required and 200 is recommended).

While most regulations and guidelines are subject to criticism for their "minimum-maximum" effect, the foregoing regulations should be adopted as minimum standards by the laboratory and clinical community and all parties should work toward the implementation of higher standards to avoid the negative impact of interpretation errors.

Proficiency testing and quality control

Most responsible laboratories utilize recognized quality control and participate in proficiency testing. While proficiency testing plays a role in monitoring laboratory performance, experience in the United States with AFP proficiency testing programs reveals that problems exist with (1) methods of reporting, (2) cutoff levels utilized for interpretation, (3) comparability of units, (4) differences in performance characteristics of currently available reagents, and (5) erratic assay performance. The Centers for Disease Control reported results from experienced AFP laboratories showing that "In interpreting whether a sample was low, or normal or abnormally high for AFP, we observed that an 80% or greater consensus was achieved on only 6 of the 10 survey samples because borderline samples were included in the survey."⁵

Recently an AFP quality assessment scheme serving 76 AFP laboratories in the United States had to be informed by participants concerning large numbers of pregnancies with low maternal serum AFP values (a reagent problem which had been observed and published 2 years earlier).^{6,7} Once the quality assessment scheme was alerted to the problem, it took steps to document and evaluate the underlying causes of the problem. This same quality assessment scheme reported results from one AFP laboratory (after 1 year of performance) showing severe assay drift with a steady downward drop in values over a year (an assay problem about which Food and Drug Administration authorities were cautioned in 1981).⁸ No information on corrective action taken (if any) during that year to either remedy the problem or prevent its negative impact on interpretation accompanied the report.

No proficiency test program can monitor all important aspects of laboratory performance. However, programs offering proficiency testing should take advantage of recommendations concerning potential AFP assay problems that have been recognized and reported by pilot maternal serum AFP experiences. Additionally, it is the AFP laboratory's responsibility to ensure that superior quality control and intralaboratory performance monitoring, beyond the scope of currently available proficiency testing, is conducted. Such monitoring can best be accomplished in a high-volume setting.⁹ Programs evaluating substantial numbers of screening samples per week (>400) will be capable of (1) monitoring the percentage of screening samples falling either above or below the normal range, (2) analyzing multilevel quality control samples in a repetitive fashion throughout large assays so as to identify intra-assay and interassay drift, and (3) utilizing patient data periodically plotted against established cutoff levels as a further alert mechanism indicating shifts in laboratory values, potentially resulting in erroneous interpretations.

These concepts are not new. Experienced pilot maternal serum AFP screening programs communicated to United States federal health authorities (Food and Drug Administration) the need for such monitoring of AFP assays 5 years ago, yet newly established AFP proficiency test programs approach the assessment of laboratory performance with a protocol that does not attempt to monitor for important aspects of AFP assay variability.

The complexities of prenatal AFP screening and the need for accurate interpretive reporting demand that the medical community no longer accept the misguided notions that laboratories merely quantitate AFP in maternal serum and amniotic fluid and that proficiency testing should simply monitor quantitative comparability. Proficiency testing should adequately monitor the "real world" of AFP testing and screening. Actual screening and diagnostic dilemmas should be simulated in order to gauge laboratory proficiency. These simulations should require laboratories to perform not only in the area of AFP quantitation but also in areas of ancillary diagnostic testing such as fetal blood analysis and acetylcholinesterase evaluation and most important an adequate assessment of the laboratory's interpretive accuracy must be made.

In terms of maternal serum AFP screening, however, it is our view that proficiency testing must extend to include an assessment of laboratory ability to provide specific risk data to physicians and patients (risk reporting will be addressed in detail in a later section).

Recent proficiency testing information has documented unique difficulties encountered when laboratories perform AFP assays. A report from one quality assessment scheme states¹:

A number of laboratories have very serious problems performing their AFP assay, as indicated by highly erratic performance. The poor performance does not result from use of inadequate assays since the kits used by these laboratories do well in other people's hands. It is of some concern that two of the poor performers have been screening for some time, indicating that experienced laboratories do not necessarily perform well.

Commercial laboratories, with one exception, do not make screening recommendations but instead provide reference data to physicians for interpreting AFP values. Examination of these reference data reveals that in some cases suggested screening cut-offs are reasonable, but grossly in error for others. It has been impossible to assess how reliably AFP values are interpreted and applied by individual physicians under these conditions.

Expression of assay results as MOM's for interpretation is more variable than the AFP values themselves.

A final report currently in preparation will stress this issue of improper reference data and its strong adverse effect on screening performance.

There are problems and pitfalls in maternal serum AFP screening, even in experienced laboratories. These problems may relate directly to the inadequate establishment of a normative data base and lack of attention to recommended methods for monitoring assay quality. In addition, inappropriate diagnostic-type proficiency assessment of a complex screening process will not, in our view, serve to bring about improved quality in screening. The screening process should define risks in individual pregnancies rather than produce pseudodiagnostic information expressed in terms of quantitative units of measurement, and proficiency testing should assess the laboratories' capabilities to produce such risks.

Risk reporting

Experience in our maternal serum AFP screening program has shown that the quantitative expression of AFP levels in maternal serum [nanograms per milliliter, International Units per milliliter, or multiples of the median (MOM)] is of problematic clinical use to physicians. Such units do not adequately express the degree of risk in the pregnancy under evaluation. Conventional, quantitative expressions have a place in diagnostic (amniotic fluid) AFP testing, but since the purpose of maternal serum AFP screening is to identify a subgroup of pregnancies that are at increased risk, pseudodiagnostic forms of reporting are misrepresentative of risk in a screened pregnancy.

Important variables have been identified that influence the interpretation of a specific maternal serum AFP quantitative level.¹⁰ These include geographic variation in the prevalence of NTD, racial differences in both the prevalence of NTD and the expected maternal serum AFP level at a given week, maternal conditions such as insulin-dependent diabetes and perhaps hypertension, and finally a widely circulated (and in our opinion invalid) suggestion that maternal weight adjustments should influence reports on individual maternal serum AFP levels.

Since the prevalence of open NTD may vary geographically by a factor of 10 within the United States, pregnant women with identical maternal serum AFP levels may vary tenfold in their risk for open NTD, depending on geographic location. This factor, taken alone, should conclusively establish that reporting AFP screens in conventional, quantitative expressions is insufficient.

It is our opinion that clinically meaningful expressions of screening results, in terms of patient-specific risk and further in terms of quantitative risk units, should be used. The Centers for Disease Control have analyzed this issue and produced a carefully planned model utilizing Bayesian analysis and incorporating all necessary variables into a laboratory's established data base in order to produce specific patient risk profiles

for multiple gestation, anencephaly, open spina bifida, and ventral wall defect.¹⁰ The authors of the Centers for Disease Control report state: "This adjustment methodology provides a straight-forward means for clinical laboratories to report results of assays of maternal serum alpha-fetoprotein in terms of predicted risks, to facilitate understanding by the physician and patient of the clinical meaning of the results of maternal serum alpha-fetoprotein testing."¹⁰

Among the important variables influencing the degree of risk for NTD are specific racial differences in the prevalence of open NTD as well as apparent variation in the maternal serum AFP levels among races. Patient maternal serum AFP-related risk for open NTD should be adjusted to account for such differences. The Centers for Disease Control report a range of prevalence for open spina bifida in pregnancies in white women of 2 to 8 per 10,000 while in pregnancies in black women the range is from <1 to 3 per 10,000. Furthermore, maternal serum AFP levels in unaffected black pregnancies are reported to be 10% higher than those in Oriental, white, and Hispanic pregnancies, suggesting that AFP levels in black mothers be multiplied by a factor of 0.9.

Since maternal diabetes has a depressing effect on maternal serum AFP levels, this is an additional factor that laboratories should consider in interpreting quantitative maternal serum AFP levels. It has been reported that insulin-dependent diabetic patients demonstrate maternal serum AFP levels in unaffected pregnancies that are 60% lower than those found in the general pregnant population. Furthermore, a significantly higher prevalence of open NTD has been reported in the diabetic population (approximately 35 per 10,000 pregnancies). An insulin-dependent diabetic pregnancy with a maternal serum AFP level of 1.5 MOM (clearly within the normal range) may demonstrate an overall risk for open spina bifida, anencephaly, and ventral wall defect of 1/17. Conventional quantitative reporting in such a case could be extraordinarily misleading.

Variables such as race, geography, and maternal conditions are not, however, the sole basis for considering improved modalities for conveying maternal serum AFP screening information. Use of diagnostic terminology or terminology that implies abnormality (for instance, standard deviation or MOM) is inappropriate. Screened subgroups of patients should not be considered or informed that they are abnormal but rather at increased risk. As such, they should be counseled to weigh screening information, that is, increased risks, within the context of: (1) risks of the relevant population and (2) the additional risks of diagnostic evaluations that may follow. The use of a standard deviation or MOM expression within the screening, counseling, and decision-making process is problematic. In our

Table I. Comparison of decision-making process in a hypothetical black patient, under 35 years of age, resident of Northeast United States

Reporting system	Report to physician	Interpretation	Patient-specific risk	Risk of amniocentesis	Patient decision
MOM	2.5 MOM	Significant elevation, increased risk for NTD, VWD	≥5% for all gravidas ≥2.5 MOM	1/200	Amniocentesis elected
Risk	Patient-specific risk; 1/439 for NTD, VWD	Low risk	1/439	1/200	Amniocentesis declined

VWD = Ventral wall defect.

opinion, such data should not reside in the patient's chart.

Professionals conveying genetic counseling information to patients undergoing maternal serum AFP screening should consider whether they possess sufficient specific information on which to counsel patients. For example, how differently would patients be informed and counseled if patient A demonstrated a maternal serum AFP level of 2.5 MOM, while patient B's level was 3.5 or 4.5 MOM or any gradation between? The need for and utility of patient-specific risk reporting in maternal serum AFP screening are raised by such questions.

Without such risk reporting, decisions on further testing will be of a binary nature (<2.5 MOM = no, >2.5 MOM = yes). Counseling under these conditions may be compared to patient notice (either benign or alarming) rather than accurate, clinical information giving patients the opportunity to weigh risks. Additionally, responsibility for decision-making may be inadvertently retained by the professional counselor (whomsoever that may be) under the binary (MOM) system rather than where it should appropriately reside, with the patient and family.

The patient chart should indicate that clear, nondirective counseling information was provided. Physicians (and counselors) should reflect on how a decision to undergo amniocentesis may be made in the case example found in Table I.

Recognizing that pregnant women with identical levels of AFP may face significantly different risks for open NTD, there is need to develop quantitative units of measure that incorporate all essential variables bearing on accurate interpretation of patient-specific risk. Without such risk units a physician receiving identical quantitative AFP levels for two patients whose risks vary significantly, faces a considerable dilemma.

Patient-specific risk units are based on patient risk for open NTD and ventral wall defect after AFP evaluation. The Centers for Disease Control have suggested that patient risk be assigned to one of three categories (Table II).

Calculation of risk units from patient risks based on

these risk categories would occur as follows: The upper limit of the low-risk range (1 in 401) equals 99 risk units; the upper limit of the intermediate risk range (1 in 101) equals 149 risk units. Because of the reported association of low levels of AFP with trisomy, we have defined 0 to 49 risk units as the "low AFP" range. Patient-specific risk of 1 in ≥10,000 for NTD and ventral wall defect is equivalent to 50 risk units.

Given the above relationships between patient-specific risks and risk units, the formulas used to calculate specific risk units are as follows*: For low risk, risk units = $15.2 \ln(\text{patient risk}) + 190$. For intermediate and high risk, risk units = $35.89 \ln(\text{patient risk}) + 315.04$. The constants in these equations (15.2, 190, 35.89, 315.04) were derived as follows: Risk units = $a \ln(\text{patient risk}) + b$. For low risk, 99 risk units = $a \ln(1/401) + b$; 50 risk units = $a \ln(1/10,000) + b$, solving for a and b , $a = 15.2$ and $b = 190$. For intermediate and high risk, 100 risk units = $a \ln(1/400) + b$; 149.4 risk units = $a \ln(1/101) + b$, solving for a and b , $a = 35.89$ and $b = 315.04$.

To generate risk units, laboratories will need: (1) adequate and independently derived normative data, (2) prevalence rates of open spina bifida, anencephaly, and ventral wall defect by geographic location and race, (3) distributions of AFP for open spina bifida, anencephaly, and ventral wall defect, and (4) patient data, including AFP level, gestational age, race, and geographic location.

Risk units may be computed by using the methodology recommended by the Centers for Disease Control to compute risks based on Bayes theorem¹⁰ and the formulas for calculating risk units, from patient-specific risks as described above.

It should be emphasized that use of risk units applies specifically to maternal serum AFP screening. Use of these meaningful quantitative risk units with a uniform reference range regardless of AFP reagents used will improve reporting between the screening laboratory

*The $\ln$ function is introduced to map the broad range of patient-specific risks to the above-defined risk units.

Table II. Patient risk categories

<i>Risk category</i>	<i>Risk</i>	<i>Risk units</i>
Low	$\leq 1/401$	50-99
Intermediate	$\geq 1/400$ but ≤ 101	100-149
High	$\geq 1/100$	≥ 150

and physician and from physician to patient. Additionally, different courses of action (involving invasive testing such as amniocentesis), taken in different cases in which identical AFP levels were found, are now justified in the physician's patient chart. In time, perhaps this form of risk quantitative reporting will be found to facilitate a system of improved proficiency testing for maternal serum AFP screening laboratories.

Maternal weight

Physiologic or statistical factors that may influence detection efficiency in maternal serum AFP screening are of obvious importance. It has been suggested that an inverse relationship exists between maternal blood volume (estimated by maternal weight) and the concentration of maternal serum AFP. Some have recommended that screening programs should adjust maternal serum AFP levels and interpretations should be based on maternal weight.¹¹⁻¹³ Such adjustments based on statistical criteria involve the implicit assumption that women of low weight have the same likelihood of NTD despite their characteristically higher maternal serum AFP values. It has been observed, however, in pregnancies with open spina bifida, that maternal weight is found to be approximately 1.6 kg less than expected.¹²

Our assessment of maternal weight and maternal serum AFP levels in 5740 pregnancies confirms an inverse relationship (Table III). However, our analysis indicates that had maternal weight correction been incorporated into the screening of this large group by any of three published procedures, a larger proportion and different group of pregnancies would have been considered at increased risk for NTD and a significant reduction of NTD detection efficiency would have occurred. It appears that the screening process would be negatively affected by the implementation of published maternal weight adjustment recommendations.

Low maternal serum AFP and chromosomal trisomy

A newly developing area within maternal serum AFP screening is based on the retrospective observation of a correlation between fetal trisomy and lower than expected maternal serum AFP levels.⁷ This finding, which should be considered investigative until results from prospective studies are published, links for the first time AFP evaluations with conventional forms of detection

Table III. Maternal weight and maternal serum AFP

<i>Weight class (pounds)</i>	<i>Median maternal serum AFP (IU/ml)</i>	<i>Median maternal serum AFP (MOM)</i>
<111	29.030	1.23
111-140	26.415	1.08
141-170	23.947	0.96
>170	21.633	0.83

of human genetic anomalies (for instance, chromosomal analysis). In that much of human genetics reporting is based on risks, it is not unusual to note that this area of AFP screening, even in its early investigative stages, has found the method of risk reporting very useful.

Combining age-related risk for Down syndrome with low maternal serum AFP risk for this same malformation may represent an important advance in the identification of some portion of the 80% of Downs cases that occur in pregnant women under the age of 35. Based on retrospective data some have advocated routine screening for Down syndrome with the use of low maternal serum AFP levels.¹¹ This proposal suffers from a lack of scientific knowledge that would be derived from controlled and prospective evaluations similar to those conducted in the late 1970s correlating elevated maternal serum AFP with open NTD. Furthermore, unlike NTD screening, which has demonstrated excellent capability in detection efficiency, it has been estimated that low maternal serum AFP screening for fetal trisomy in young mothers may alert clinicians and families to perhaps an additional 20% of cases.

Additional dangers that may arise from premature initiation of low maternal serum AFP screening relate primarily to the areas of AFP reagent specificity and physician liability in the event of the birth of an unscreened and/or undetected trisomy.

In the United States, use of different commercially available AFP reagents results in variable proportions of normal pregnancies with maternal serum AFP levels falling below specific cutoffs. This concept was recognized in the original contribution, correlating low maternal serum AFP levels with fetal trisomy.⁷ For example, one AFP reagent kit evaluating 100 normal 17-week maternal serum specimens may yield 15% falling below 0.5 MOM while a second AFP kit evaluating the same 100 specimens will produce 5% falling below 0.5 MOM. Under the conditions of routine low maternal serum AFP screening, the effect on the number of amniocenteses can be sizable, depending on the reagents in use in a particular laboratory. Laboratories that inappropriately designate large numbers of patients at risk for trisomy will generate clinical data from which the conclusion may be drawn that this aspect of ma-

ternal serum AFP screening is not viable. Before low maternal serum AFP screening is implemented, the laboratory and clinical communities should take steps to make certain that patients are aware of all the facts.

Premature initiation of low maternal serum AFP screening may also serve to create a problematic medico-legal environment in which the birth of any un-screened trisomic fetus might be viewed as worthy of legal action. This problem becomes particularly difficult when one considers that the estimate for successful identification with low maternal serum AFP screens may be as low as 20% of trisomic cases in young, apparently healthy families.

In the absence of prospectively generated data leading to accurate risk profiles that could be made available to patients for decision making and a major educational effort aimed at both the professional community and the public, we believe that routine clinical low AFP screening places obstetricians at additional, unwarranted risk. A prudent step toward implementing low maternal serum AFP screening would be to have obstetricians join with regionally established investigations on the association of low maternal serum AFP levels and trisomy to scientifically validate this field of prenatal screening.

Comment

Maternal serum AFP screening, a complex interdisciplinary process that we initiated in July of 1976 and conducted for 10 consecutive years, is now expanding toward 3.5 million annual United States pregnancies. With experience, lessons have been learned and used to improve performance. Our recommendations have been reported in the scientific literature and submitted to federal and state health authorities. Nevertheless, the implementation of population-based maternal serum AFP screening is proceeding, in many instances, contrary to recommendations on: (1) normative data, (2) quality control, (3) proficiency testing, and (4) patient-specific risk reporting. Instead problematic adjustments involving maternal weight have been adopted and problematic fragmentation, such as the entry of many small laboratories into the field without first having developed sufficient normative data, has occurred.

Fortunately some state health authorities have taken up the challenge and issued regulations designed to

foster public health. We have identified important issues and potential pitfalls in order to improve maternal serum AFP screening and prevent negative public health outcomes. Maternal serum AFP screening, as the first prenatal population-based screening process, continues to face great challenges. Proper information and education concerning this biomedical field will help to ensure its safe and effective application and successfully meet the challenge.

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Bilateral breast cancer: The frequency of undiagnosed cancers

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The risk of developing cancer in the contralateral breast is five to seven times the risk of cancer occurrence for the normal female population. In patients in 365 consecutive operations, 15 frankly invasive cancers and four metastatic lesions were found in the contralateral breast as well as an additional 28 lesions in situ, totaling 13%. An additional 30% of the patients had severe dysplasia with an unknown potential for becoming cancerous. Only less than one third of the cancers in the contralateral breast were symptomatic (by palpation or mammography or both) but not recognized at the time of primary treatment. The majority of lesions in the contralateral breast are due to a second primary growth, and only a minority is metastatic. The results of 203 bilateral operations consisting of a modified subcutaneous mastectomy with axillary lymph node dissection and radiation are presented; this cosmetically acceptable procedure reduces the number of cancers in the contralateral breast to an incidence of 1% to 2%. (AM J OBSTET GYNECOL 1986;155:247-55.)

Key words: Breast cancer, bilateral, risks, modified subcutaneous mastectomy

The optimal surgical procedure for treatment of breast cancer has become a matter of dispute. However, a review of the literature makes it obvious that radicalness has been reduced and breast-preserving procedures have been preferred over the last decades.

Breast cancer metastasizes early. This is explained by the multiplying time of tumor cells, which is in a magnitude of approximately a hundred days. A tumor of 1 cm in diameter which is barely palpable has therefore been developing for 6 to 8 years.^{1,2}

Breast cancer is also a disease of multicentric origin. This is underlined by the rather large number of cancers that develop in the contralateral breast. Studies performed during the last 15 years revealed an incidence of 12% to 21% for cancers in the contralateral breast. However, noninvasive tumors also were included in this figure by some authors. Manifestation of a latent tumor can be expected in 2% of the cases up to 6 years after the detection of the first cancer³ and an additional 6% can be expected in the next 20 years.¹

The risk for a woman to develop cancer in the con-

tralateral breast is 5 to 7 times the risk of cancer developing in the normal female population. The risk is higher in premenopausal patients (13.3% compared to 3.5% of postmenopausal).³ Urban⁴ pointed out the danger with the statement that "the most frequent antecedent of cancer in one breast is the history of having had cancer in the opposite breast."

Our group began in 1974 to replace the modified radical mastectomy with lymphadenectomy by a subcutaneous mastectomy with lymphadenectomy and radiation. Required was the removal of 95% of the glandular part of the breast, which in turn justified the term "radical." Secondary augmentation by silicone implants under the skin and bilaterality of the operation were performed after the request of the patient. The 10-year results of removal of tumors up to 4 cm (T₂) regarding survival, freedom from metastatic growth, and the rate of local metastasis have been presented elsewhere and were better than 80%.² However, cosmetic results after the augmentation procedure were hampered because of thickening of the connective tissue forming the capsule. The radicality of the subcutaneous mastectomy was therefore reduced to approximately 80%, thereby preserving fat which allowed for mobilization of fat flaps and augmentation without foreign material. Glandular tissues with connective tissue septa (Cooper's ligaments), which are close to and run into the panniculus adiposus and overlying breast skin, remain in situ. Thus the glandular, connective, and adi-

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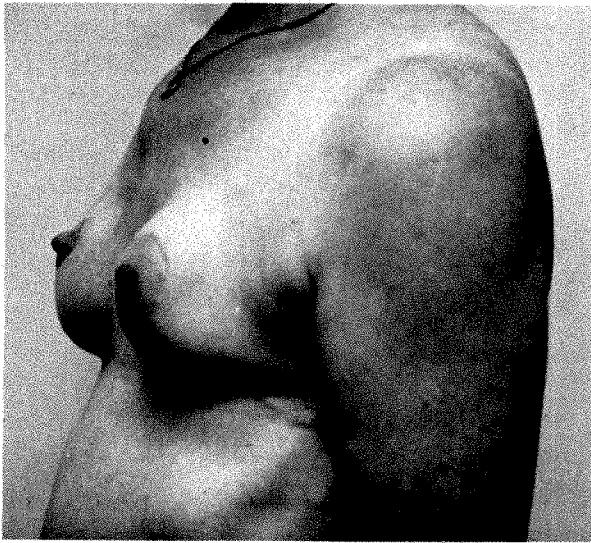


Fig. 1. Cosmetic result after modified subcutaneous mastectomy with reconstruction.

pose tissue left behind is used to construct a smaller breast by a technique slightly modified from the reduction mammoplasty we described earlier.

This procedure is called a "partial or modified subcutaneous mastectomy with lymphadenectomy and simultaneous reconstruction."^{8,9} Because of the very acceptable cosmetic results 98% of the patients approved of the bilateral procedure. Fig. 1 represents the cosmetic result as an example. This enabled us to gain material for comparative histopathologic examination of both breasts.

In this paper we report the incidence of undiagnosed small and large cancers in the contralateral breast detected at the time of primary treatment by examining the contralateral breast.

Material and methods

Patients. The breast tissue simultaneously removed from both breasts by the radical or modified operation was examined consecutively in patients with breast cancer. When cancer was found in the contralateral breast, the chart was reexamined for symptoms of cancer in the contralateral breast which were possibly overlooked.

The modified procedure was performed in 203 patients, and for this group data were prospectively collected.

Histopathology. Biopsy specimens were freshly cut into laminae, and the adipose tissue was separated from the specimens. Areas of focal changes, such as papillomas, sclerosis, and changes in staining properties of the breast tissue, were examined microscopically in fresh frozen sections. If a malignancy was diagnosed, the operation was immediately extended on both sides.

Table I. Criteria for classifying cancers in the collateral breast as a second primary tumor or as metastatic

Criteria	Primary	Metastatic
Growth pattern	Focal, centrifugal	Diffuse
Lymphangiosis	Facultative	Obligatory
Tumor border	Dysplastic, in situ	Inert
Differentiation	Facultative	Identical
Topographic evaluation	Arbitrary in the organ	Pass boundary of the organ along lymphatic and/or vascular spread

The fresh-frozen biopsy blocks, as well as the remaining tissue, were fixed in formalin for 12 hours after the operation, and multiple laminae were cut, embedded, and stained with hematoxylin. The breast tissue was examined stepwise in numerous sections with one biopsy specimen yielding about 20 tissue blocks. All specimens were classified by the pathologist (H. N.), in cooperation with the surgeon in charge (F. K. B.). The more detailed evaluation of dysplasias and the final determination of the degree of mastopathy were exclusively based on tissue sections.

Criteria for histologic classification. Our histopathologic criteria for dysplasia are based on the concept of progressive dysplasia of the breast tissue, as described originally by Aschoff and extended semiquantitatively by Wellings et al.,¹⁰ and are shown in Table I and described in detail elsewhere.

Results

Of 365 patients with cancers in the ipsilateral breast 15 had frank invasive cancers in the contralateral breast and an additional 28 TIS (according to the staging of the World Health Organization) lesions. This represents an incidence of 13%. There were 111 (30%) cases showing severe dysplasia or lesions considered precancerous (Table II).

The age distribution is shown in Table III. There were twice as many cancers in the contralateral breast before menopause as after. This is in contrast to the primary tumor in the ipsilateral breast, of which nearly 70% developed postmenopausally. It is of significance that three of the four metastatic lesions in the other breast were found in young patients. The age distribution in the group of dysplastic lesions is homogeneous. Metastatic lesions were found in patients with larger tumors in the ipsilateral breast (Table IV) when secondary primary tumors were already seen in earlier stages.

Second primary tumors in the contralateral breast (Fig. 2, A and B) were identified in half of cases with negative, axillary lymph node status. This is in contrast to the metastatic lesions (Table V). Dysplastic lesions

Table II. Incidence of cancers in ipsilateral and contralateral breast at the time of primary treatment (radical and modified subcutaneous mastectomy)

	Ipsilateral breast	Contralateral breast				• Severe dysplasia or precancerous lesion
		Secondary cancer (T ₁)	Primary cancer (TIS)	Metastasis	Σ	
n	365	15	28	4	47	111
%	100	4	8	1	13	30

Table III. Age distribution of cancers in the contralateral breast (modified subcutaneous mastectomy, n = 203)

Age (yr)	n	Secondary cancer (≥T ₁)	Primary cancer (TIS)	Metastasis	Σ		Severe dysplasia or precancerous lesion	
					n	%	n	%
25-34	3	—	—	2	2	1	1	1
35-44	14	3	4	1	8	4	3	2
45-54	43	3	6	—	9	4	10	5
55-64	82	1	5	1	7	3	33	16
65-74	50	1	2	—	3	2	13	6
75-84	11	—	—	—	—	—	2	1

Table IV. Stage of cancers in the ipsilateral breast related to number of tumors in the contralateral breast (modified subcutaneous mastectomy, n = 203)

Ipsilateral breast		Contralateral breast						
Stage	n	Secondary cancer (T ₁)	Primary cancer (TIS)	Metastasis	Σ		Severe dysplasia or precancerous lesion	
					n	%	n	%
T ₀	2	—	2	—	2	1	—	—
T ₁	64	3	3	—	6	3	18	9
T ₂	95	3	7	1	11	5	31	15
T ₃	31	2	3	2	7	3	10	5
T ₄	11	—	2	1	3	2	3	2

are evenly distributed. The histologic type of cancer is also evenly distributed with possible, but not statistical, significant medullary cancers (Table VI). It is expected that twice the number of cancers in the contralateral breast were related to a grade III, and therefore an undifferentiated, tumor (Table VII). Palpation was positive in 48 of the 203 cases; however, only 10 were associated with cancers, 20 were premalignant lesions, and 18 were false positive. In 155 women without palpable tumor in the contralateral breast, 19 cancers and 42 premalignant lesions were found. Mammographic results were not much better. Of 36 mammographic positive findings, 13 were false positive and 22 cancers were missed by the procedure.

Only in four patients was the lesion in the contra-

lateral breast considered metastatic. An example is presented in Fig. 2, A and B. The majority therefore had a primary cancer. Figure 3, A and B, represents an example of cancers with different structure in both breasts and therefore considered to be second primary growth in the collateral breast.

Comment

Our data demonstrate that cancers in the collateral breast requiring treatment were present in 13% of cases. The number of cancers which would have developed from precancerous lesions, which were found in an additional 30%, is open for discussion. By using identical criteria for dysplastic lesions, we have performed subcutaneous mastectomy as a prophylactic op-

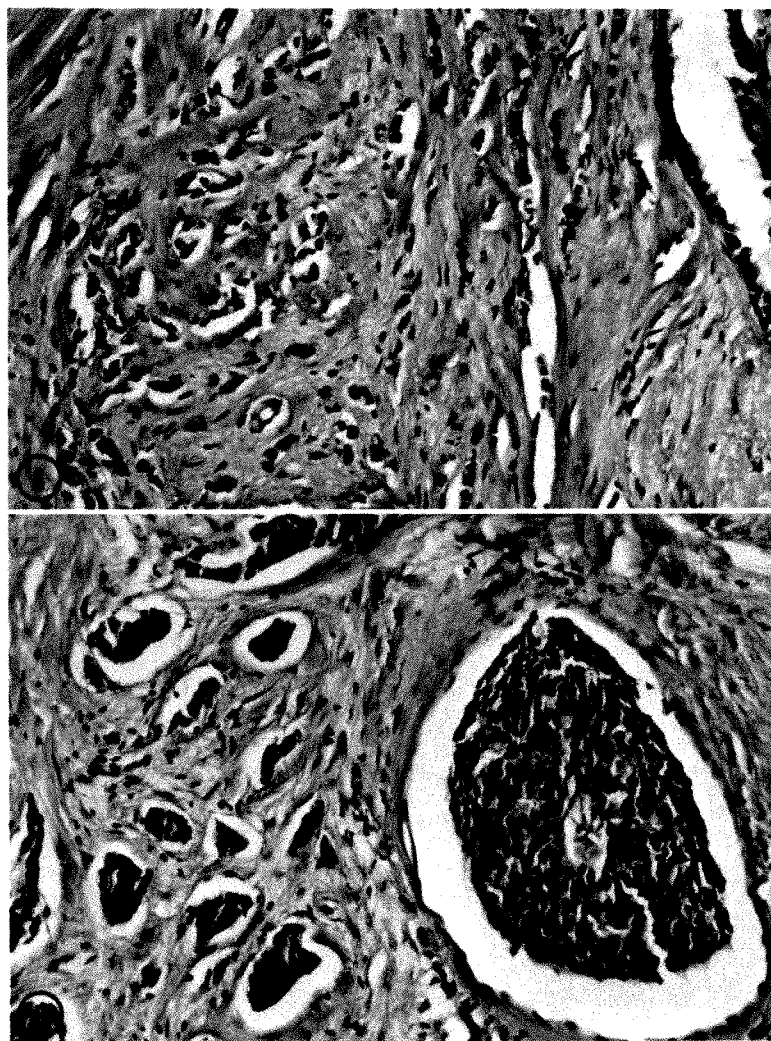


Fig. 2. A, The tumor was 2.5 cm in diameter of the right breast. Lobular cancer was classified as grade III because of satellite tumors and severe lymphangiosis. B, Cancer of the left side 2 years after primary procedure. Lymph nodes were identified on this side, and the operation performed. There was now a diffuse carcinosis tumor, stage $T_{R1L1}N_{R1L1}M_0$.

Table V. Lymph node status of ipsilateral breast related to tumors in the contralateral breast (modified subcutaneous mastectomy, $n = 203$)

Ipsilateral breast		Contralateral breast						
Lymph node status	n	Secondary cancer ($\geq T_1$)	Primary cancer (TIS)	Metastasis	Σ		Severe dysplasia or precancerous lesion	
					n	%	n	%
N ₀	113	4	9	1	14	7	29	15
N ₁	36	2	4	1	7	3	12	6
N ₂	54	2	4	2	8	4	21	10

eration. This will be reported in another paper. We found an incidence of 25% latent microscopic clinically unknown cancers. This value corresponds well with similar data from the literature. This led us to assume

that more cancers can be expected to develop in the contralateral breast in the next 10 years.

It is difficult to compare our data with those of previous reports. There are more than 40 papers in the

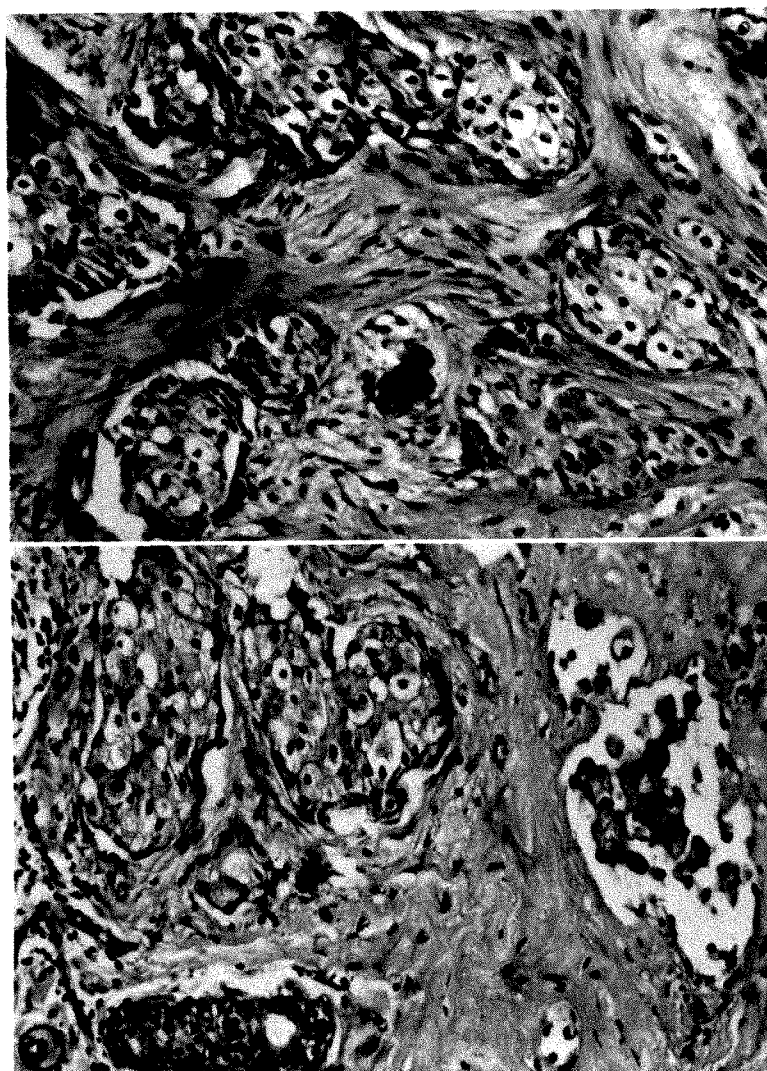


Fig. 3. A, Lobular cancer (scirrhous), 1.5 cm in diameter, in left breast. B, Secondary primary tumor, 2 cm in diameter, in right breast. Final tumor stage, T₄L₁N₀M₀. Note the ductal differentiation of the tumor in the contralateral breast.

literature reporting the incidence of cancers in the contralateral breast. Data are, however, difficult to compare, because the cancer in the contralateral breast has been detected by different methods. These include clinical symptomatology (including palpation or mammography), routine biopsy of the other breast at the time of primary treatment for the ipsilateral breast (as requested by some surgeons), or finally by retrospective evaluation of data in the literature. The differences in evaluation of cancers in the contralateral breast explain the large discrepancy concerning incidence, which ranges from 0.2% up to 24%. The addition of precancerous lesions, with a lack of standardized histopathologic evaluation, added to the large variance of results.

Our material represents the largest group of patients in whom the number of cancers in the contralateral breast are identified at the time of primary treatment

by subcutaneous mastectomy. Our numbers represent the minimum number of cancers in the contralateral breast. The presence of undetected small cancers cannot be excluded, since we were not able for technical reasons to perform serially the entire tissue mass.

Many surgeons have attempted by various means to reduce the incidence of contralateral breast involvement. The prophylactic operation of the contralateral breast was attempted as early as 1920. Pack¹¹ again advocated bilateral mastectomy in 1951. In contrast, Herrmann¹² indicated that, if routine mastectomy had been performed, 47% of his patients would have undergone a useless mastectomy. Even when patients with special risk factors underwent mastectomies, this was considered as an overtreatment.

With radical subcutaneous mastectomy we achieved results that were cosmetically superior to modified rad-

Table VI. Histologic type of cancers in the ipsilateral breast compared with tumors in the contralateral breast (modified subcutaneous mastectomy, n = 203)

Ipsilateral breast		Contralateral breast						
Histologic type	n	Secondary cancer ($\geq T_1$)	Primary cancer (TIS)	Metastasis	Σ		Severe dysplasia or precancerous lesion	
					n	%	n	%
Lobular	93	4	5	2	11	5	35	17
Ductal	44	2	4	—	6	3	9	4
Tubular	11	1	1	—	2	1	3	2
Signet-ring carcinoma	6	1	—	1	2	1	1	1
Medullar	12	—	5	—	5	2	—	—
Scirrhus	5	—	—	—	—	—	2	1
Lobular plus ductal	18	—	2	1	3	2	4	2
Lobular plus tubular	8	—	—	—	—	—	6	3
Ductal plus tubular	2	—	—	—	—	—	—	—
Tubular plus medullar	1	—	—	—	—	—	1	1
3-4 types of tissue	3	—	—	—	—	—	1	1

Table VII. Grade of malignancy in the ipsilateral breast compared with tumors in the contralateral breast (modified subcutaneous mastectomy, n = 203)

Ipsilateral breast		Contralateral breast						
Grade	n	Secondary cancer ($\geq T_1$)	Primary cancer (TIS)	Metastasis	Σ		Severe dysplasia or precancerous lesion	
					n	%	n	%
I	15	—	2	—	2	1	4	2
II	79	2	4	1	7	3	22	11
III	109	6	11	3	20	10	36	18

ical mastectomy, because the areola and the nipple are preserved. The 10-year survival rate is identical to that of more radical procedures, and in several thousands of such operations, not a single incidence of local recurrence was observed in the areola after the breast was radiated. However, augmentation with silicone implants has a high cosmetic failure rate because of capsular contraction. This in turn excludes bilateral radical subcutaneous mastectomy as a prophylactic bilateral operation. The lesser radical approach allows breast construction from fat flaps. It was expected to reduce the incidence of cancer in the contralateral breast to approximately 1%, which was found over a time period of 4 years. In both patients tumors were removed by tylectomy. This lower incidence of breast cancer seems to offer an acceptable compromise for reduction of breasts larger than 300 gm.

Modified subcutaneous mastectomy with fat flap augmentation seems to provide an acceptable compromise between good cosmetic result and prophylactic prevention of cancer in the contralateral breast.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. GEORGE W. MITCHELL, San Antonio, Texas. The appearance on this program of a report on the surgical management of breast disease must be considered a landmark, since there has been no precedent in modern times for an essayist from this podium to stray so far beyond the surgical boundaries that have been set for gynecologic practice. Whether it will also be a turning point remains to be seen. Speculation as to the reason for this apparition suggests the following possibilities: (1) the originality and daring of the proposed operation, (2) Dr. Beller's well-known charm and persuasiveness, (3) the desperation of the secretary for papers, (4) the recent realization by gynecologists that the breast is primarily a female organ, and (5) two widely publicized papers in the *New England Journal of Medicine*^{1,2} which suggest that the technical aspects of the breast surgery of the future may be within the range of even the most butter-fingered surgeon. If asked, one might be tempted to answer, "All of the above"; but I think that the preferred selection is clearly the first, and I would like to thank Dr. Beller for showing us that on the continent of Europe members of our specialty are well rounded and that a single individual can be at once an accomplished hematologist, obstetrician, radical pelvic surgeon, and breast surgeon.

I cannot resist this opportunity to reiterate the obvious, a theme which has been propounded in the current put-away and the throw-away journals, deliberated by the Board, and ignored by the rank and file. We are held responsible for the diagnosis of breast disease, which involves critical decision making and legal liability, asked to manage breast problems associated with lactation, infection, endocrinopathy, and neurosis, but we are restricted by our own inhibitions from performing a procedure as simple as a biopsy. The administrative, political, and legal concerns that have been expressed about taking that next step could probably be overcome by pressure from within the specialty and from the female public, and the technique can be easily acquired and disseminated, just as the pioneers in colposcopy, laparoscopy, microsurgery, and laser surgery set themselves up overnight as experts and started giving postgraduate courses. Furthermore, we can unleash more than 200 gynecologic oncologists, who have already progressed from the pelvis to the diaphragm and who possess both the eagerness and the expertise to lead us into this new field.

I will now try to give Dr. Beller some substance to

address in his rebuttal. It is unfortunate that just when we began to talk about lesser breast cancer surgery being appropriate for lesser surgeons, Dr. Beller has inaugurated a technique, based on a form of reduction mammoplasty, which requires the skill of a plastic surgeon as well as the critical eye of an oncologist. I believe that few of us will be able to follow in his footsteps.

In the manuscript it is not clear whether the 203 breast cancer patients were all of the breast cancer patients admitted to Dr. Beller's service or whether they were selected. If they were selected, what were the criteria? For instance, were women with small breasts excluded, and, if so, how did their results compare with the others?

The speed of surgery could not eliminate all postoperative morbidity, a factor which Dr. Beller commented on in his book but not in this paper. How often did those breast flaps come apart or slough and how many patients had postoperative anesthesia of the nipple, areola, or surrounding skin? Dr. Beller has commented on the long-term morbidity associated with silicone implants as opposed to the use of fat pedicles, but implants permit resection of larger amounts of breast tissue, safer from the point of view of recurrent or new primary cancer, and do not cause fibrous capsule formation if placed below, rather than above, the pectoralis major muscle. How would these compare? Since postoperative irradiation was employed, was the cosmetic result as good after 5 years as after one? Many other questions can be raised, but the most important issue is the rebirth of the old proposal that if cancer develops in one breast, both breasts should be treated. In favor of this thesis is the fact that invasive cancer was found in 6% of the contralateral breasts, in situ lesions occurred in 14%, and severe dysplasia in 31%. The life history of in situ lesions of the breast remains unknown, although some do progress to invasion; dysplasia of the breast is a term used more often in this country by radiologists than by pathologists but presumably is synonymous with atypical hyperplasia, which is thought to be a risk factor but which has an undetermined prognosis in any individual case. From the cosmetic viewpoint a reconstructive operation on only one side often leaves a dissymmetry, which may necessitate a later plastic surgical procedure on the other.

Against the prophylactic efficacy and improved appearance must be placed the morbidity of the breast operation, the lack of a breast with normal function and sensation, and the fact that the presence of incipient breast cancers can usually be detected by mammography. Thirteen false positive mammograms out of 36 and 22 false negatives suggest that German radiologists should come to this country to learn to read mammograms just as American gynecologists should go to Germany to learn to do breast surgery. Dr. Beller has made a good case, provided that all alternatives have been presented to each patient. He has also done us a valuable service.

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DR. DOUGLAS J. MARCHANT, Boston, Massachusetts. This paper again brings up the bilaterality of breast disease. As one reviews the literature, particularly the analytic epidemiology of breast cancer, one is impressed by the fact that whatever causes breast cancer affects both breasts. Why it is expressed in one before the other is not known.

I am struck by the fact that as I see patients who I know have simultaneous cancers and who are survivors, I seldom see the lesion become overt in the opposite breast, and I wonder whether there is some difference in the biology of these contralateral cancers that has not been addressed.

Equally important is the fact that there is much disagreement about premalignant lesions. Is the macrocyst a premalignant lesion? Some think so.

What is the degree of dysplasia that we must consider?

I think all of these are issues that will be debated for many, many years.

Your operation in some cases might be considered an exercise in futility because in this country we are having trouble doing one operation on the breast. Every one is for conservative treatment, but you have neatly sidestepped this by doing, as Dr. Mitchell has pointed out, a very good technical plastic procedure.

The presence of this paper to me means that we are on the road to getting care of the breast back into the obstetric and gynecologic field, as I have urged, as you know, for many years.

I really think we should get to the curriculum committees of the medical schools. In our department we give the reproductive course on the breast. We describe the anatomy, physiology, and cancer of the breast, and it is amazing to me how many medical students send patients to our breast clinic. In other words, if we get to them at that level and let them know that we do know something about the breast, it is certainly helpful. In our residency programs many of the breast procedures are outpatient work, without a confrontation with the general surgeon, including aspiration of cysts and skinny needle biopsies, and we should be doing this. If we fail in our responsibilities, I believe that benign disease will continue to be mistreated, and worse, breast cancer will fall into the hands of the radiotherapist and plastic surgeon who are treatment oriented and not patient oriented.

I would like to ask you one or two questions about the mammography. You reported a very high false negative rate, as Dr. Mitchell mentioned. We rely very heavily on our mammography; could you give us some advice, knowing the litigious climate in this country and

the confrontation with the general surgeon, how can we get care of the breast back into obstetrics and gynecology?

DR. RAYMOND LEE, Rochester, Minnesota. I would like to compliment Dr. Beller for an innovative operation.

When our colleagues in pathology discuss premalignant lesions, a great deal of controversy may arise. We have significant concern that the patient with intraductal papillomatosis or sclerosing adenosis harbors a premalignant condition, so I would like to ask the author: Do you believe that all the patients with premalignant lesions, as you have designated, given sufficient time are destined to develop a carcinoma?

If that were the case, a considerably higher incidence than the customary 1% per year for the first 20 years would result.

Second, there are four subsets of patients in whom we are concerned about the opposite breast. We are concerned about the patient who has a family history of bilateral carcinoma of the breast. We are worried about the patient who has a carcinoma of the breast and is premenopausal. Also of concern is the patient who has multicentric carcinoma in her original operative specimen or the patient whose pathologic study reveals lobular or comedo carcinoma. Your report was of interest not only for the answers it has given but for the questions that are raised.

DR. ALFRED I. SHERMAN, Detroit, Michigan. We have been very interested in the local immunocompetence of the breast in terms of lack of metastases to the opposite breast in most cases when there are widespread metastases. Were there any patients—I did not find it in your report—who had a double lesion in the contralateral breast, such as a carcinoma in situ lesion and a metastatic lesion, or does the presence of a carcinoma in situ lesion prevent a metastatic lesion from arising in that particular breast?

DR. BELLER (Closing). The operation is standardized and performed by members of the staff who are experienced gynecologic operators but have no training in plastic surgery. Lymph node adenectomy is technically not a difficult operation. The operation is offered to all patients with breast cancers except elderly patients for whom time of anesthesia is of significance. Approximately 80% of our patients chose this type when they had the opportunity to see the results on other patients who had received the operation. The larger the breast, the better will be the cosmetic result. If the breast is very small, we perform a tumorectomy and biopsy the contralateral breast.

Slough of skin occurs in less than 2% of cases, and necrosis of the areola is in a magnitude of 2%, usually associated with large tumors.

There is no indication to believe that life expectancy, freedom of recurrence, and number of recurrences will be compounded by the operation, since we are removing more tissue than do surgeons who have published on randomized material of tylectomy (sequential mas-

tectomy and quadrant biopsy are synonymous terms). Eighty percent of local recurrences develop within the first 2 years after surgery. We have a 4-year experience to date. The fact that our local recurrence rate is very small leads me to believe that "tumor control" in our hands is very complete.

Our material differs from previous attempts in that our numbers represent the actual number of tumors in the contralateral breast at the time of primary treatment. Our definition of a TIS lesion is very rigid. The term lobular carcinoma in situ for instance is reported in our material in the group with dysplastic lesions. Nobody can predict the number which would have emerged eventually into cancer. We do not believe that we can eliminate cancer completely by the operation. Fisher et al. have shown that by radiating the ipsilateral breast the incidence of multicentric origin is reduced from 28% to 7%. In 4 years we had one such incidence but two cancers out of 200 in the contralateral breast. Both were identified early, and tylectomy with radiation was performed. I believe therefore that the operation provides a reasonable compromise between tumor prevention, local control, and cosmetic result.

Most cancers in the contralateral breast were small, which explains the failure of mammography and palpation. A tumor has to reach a diameter of 1 cm before it can be palpated. By that time it has grown for 4 to 6 years.

The breast is a reproductive organ. The only physician who has gained by training, knowledge, and understanding of the development, anatomy, function, especially in pregnancy, and endocrine procedures of the female breast is prohibited from performing any surgical procedure.

Differences in premenopausal in contrast to postmenopausal breast cancer results point to the significance of endocrine status, not to mention estrogen and progesterone receptors and their significance for treatment.

Dr. Marchant, I believe what is needed is a breast clinic in a gynecologic setting. It should be concerned not only with diagnostic problems but especially with the treatment of benign breast disease. Such treatment is in nearly all instances at present by hormonal active substances. I can assure you—competition from the surgeon will be the exception. To control the development of benign breast disease requires biopsies of various types, and this leads then to the treatment of abnormal growth.

If the breast as an organ receives more interest from the gynecologist, then the outcome will be clear. If the surgeons require hysterectomies for their training program, we should require surgery on the female breast based on confidence for ours.

Gynecologic anatomy with magnetic resonance imaging

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Magnetic resonance imaging demonstrates gynecologic anatomy not visible with other techniques. Tissue layers within and outside the uterine corpus, cervix, and vagina are defined in any plane. Such soft tissue contrast resolution is promising for the noninvasive diagnosis of disease. (AM J OBSTET GYNECOL 1986;155:255-9.)

Key words: Gynecologic anatomy, magnetic resonance imaging

Magnetic resonance imaging (MRI) is a new radiologic development that permits noninvasive examination of the female pelvis without ionizing radiation. Unsurpassed contrast resolution enables visualization of organ structure not seen with other techniques. Ultrasound imaging depends on tissue differences in

acoustic impedance, whereas computerized tomographic scanning generates an electron density map from x-ray attenuation coefficients. More morphologic information is available with MRI because the technique depends not on one but on four intrinsic tissue parameters: hydrogen density, the two relaxation times of hydrogen (T_1 , T_2), and bulk proton motion (blood flow).

Magnetic resonance images are created from the interaction of static magnetic fields, radio waves, and atomic nuclei. Protons, in water or lipid, behave like magnets when placed in a strong magnetic field. When weak radio waves of their resonant frequency pulses

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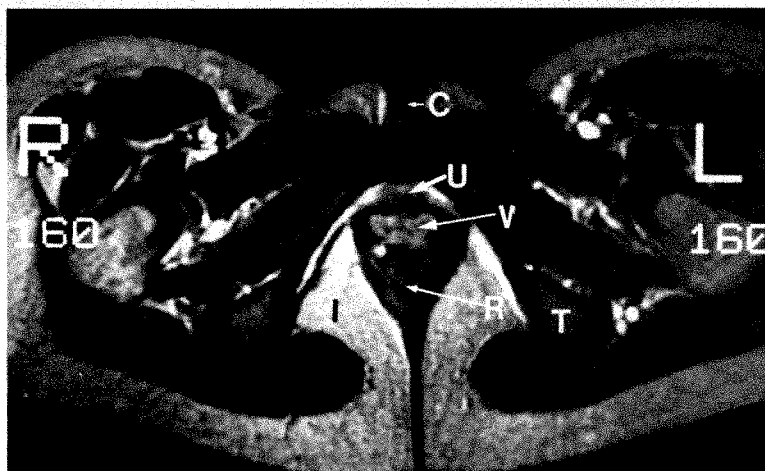


Fig. 1. Axial image (1.5 T; SE TR 3000, TE 40) through ischiorectal fossa (*I*) and ischial tuberosity (*T*). Note, from anterior to posterior, the glans clitoridis (*C*), urethra (*U*), vagina (*V*), and rectum (*R*).

are applied, the nuclei change their direction of spin and reorient in the static field. As the nuclei return to their original alignment (equilibrium) they emit radio waves of the same frequency of those that were absorbed. In a given magnetic field, the frequency of the radio waves causing resonance is specific for each nucleus; therefore superimposition of a gradient magnetic field permits spatial encoding of the signal, that is, a proton map (image).

Manipulation of the timing of the resonant frequency pulse changes the relative contribution of hydrogen density, T_1 , and T_2 such that the resultant image is "weighted." Changing the interval between successive excitations (TR) will selectively enhance tissues according to their T_1 , the rate at which nuclei realign with the static field. A short TR is insufficient time for tissues with a long T_1 to realign and therefore they generate less signal. Varying the interval between excitation and observation of the signal (TE) selectively enhances tissues according to their T_2 , the rate of loss of transverse magnetization. On long TE images, tissues with a long T_2 have larger signals and so appear brighter on the magnetic resonance gray scale. Finally, if hydrogen nuclei move through the imaged volume before data acquisition is complete, their signal can be completely lost. Consequently, at certain pulse sequences, rapidly flowing blood is signal free, hence vessels appear black.¹ In MRI, intravenous contrast is not necessary to define vascular anatomy. Choice of pulsing technique used for data acquisition varies depending on the particular tissue being studied. The most commonly used sequences today are spin echo, partial saturation, and inversion recovery.

The energy changes in magnetic resonance are enormously different from x-ray computerized tomography. One x-ray photon has at least a billion times the

energy of an electromagnetic photon concerned in nuclear magnetic resonance; hence magnetic resonance appears to be a very safe technique. With current exposure conditions, no biologic effects have been demonstrated.^{2,3} The Food and Drug Administration warns that pregnant women should not undergo imaging; however, several institutions do so with approval of their local institutional review board. Magnetic resonance can then be used when an obstetric ultrasonogram is equivocal for fetal anomalies or masses complicating pregnancy.^{4,6}

MRI is particularly well suited for gynecology because it is noninvasive, produces multidirectional images, uses no ionizing radiation, and is not impeded by gas or bone. Degree of bladder filling is unimportant. The relationship of the reproductive structures to other pelvic viscera is exquisitely displayed since fat (short T_1 , long T_2) naturally contrasts with muscle (long T_1 , short T_2). The vesicovaginal septum, the rectovaginal septum, and the levator ani can be routinely identified. The urethra can be clearly demarcated from the vagina, in contradistinction to other modalities (Fig. 1). The glans clitoridis, being erectile tissue, stands out as two linear high-intensity structures. The ovaries appear at low to medium intensity at short (T_1 -weighted) pulse sequences and blend with pelvic fat at longer (T_2 -weighted) intervals.⁷ Contained cysts, however, are usually very dark or bright depending on their serous or hemorrhagic nature. Subacute and chronic hemorrhage have a characteristic appearance, being bright on T_1 - and T_2 -weighted images. Other types of fluid are relatively dark on T_1 -weighted images and bright on T_2 -weighted sequences.^{8,9}

Sagittal T_2 -weighted images optimally display internal organ structure (Fig. 2). The uterine corpus can always be identified because of its characteristic internal

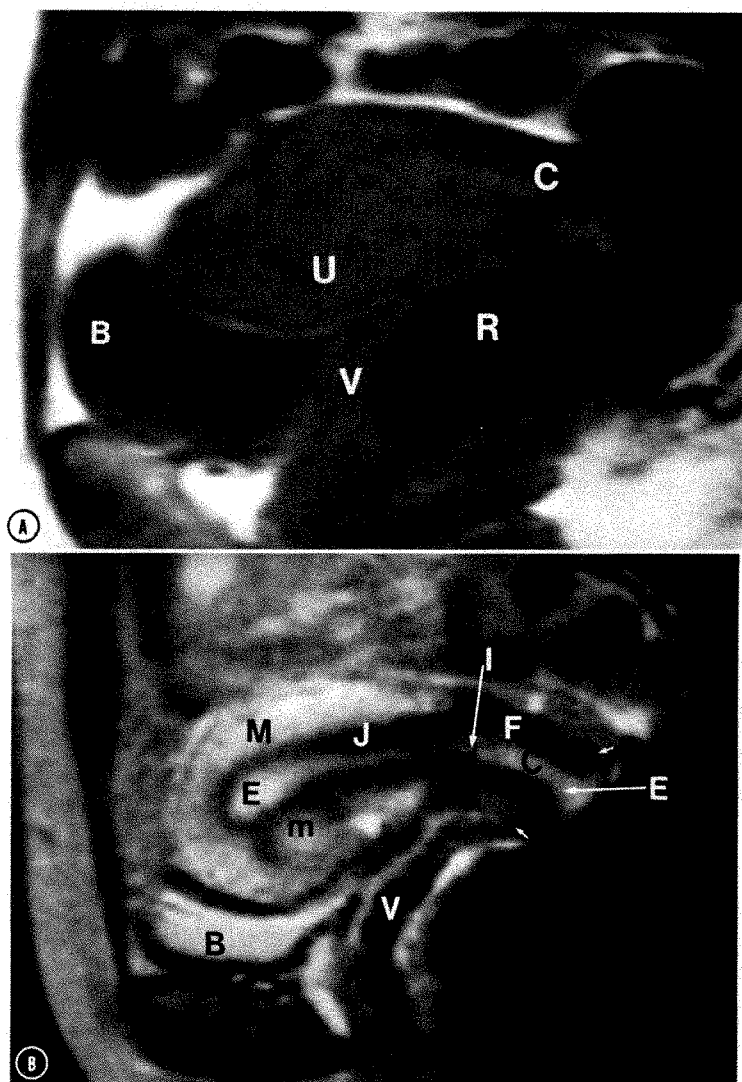


Fig. 2. **A**, Sagittal T₁-weighted image (1.5 T; PS TR 400) of pelvis demonstrating lack of tissue contrast: *B*, bladder; *U*, corpus uterus; *C*, cervix; *V*, vagina; *R*, rectum. **B**, Sagittal T₂-weighted image (1.5 T; SE TR 2000, TE 40) of a woman in the secretory phase of the menstrual cycle. Note the zonal architecture demonstrated myometrium (*M*) with a small myoma (*m*), junctional zone (*J*), endometrium (*E*), internal (*I*) and external (*E*) os, cervical canal (*C*), fibrous stroma (*F*), and vaginal canal (*V*). The anterior and posterior vaginal fornices are demarcated by *small arrows*. Note that the bladder (*B*) is almost empty.

zonal architecture. Furthermore, this architecture changes with the hormonal milieu of the woman. In premenopausal women, the endometrium appears as a well-defined high-intensity structure that increases in size during the secretory phase. In postmenopausal women or women using oral contraceptives, the high-intensity zone is much thinner, representative of atrophy.¹⁰ A surrounding dark stripe, the junctional zone, may be due to a myometrial blood flow phenomenon.¹¹ Myometrium, being smooth muscle (short T₂), is of much lower intensity than endometrium (long T₂). The origin of masses including the myometrial location of leiomyomas can be discretely stated. Distinction of sub-

mucosal versus serosal location is important since the treatment differs. Leiomyomas have no specific appearance on magnetic resonance as would be expected from their varying histologic features (Fig. 3). However, heavily calcified ones are very dark or black because of their low proton density. Visualization of individual tissue layers within the corpus enables evaluation of endometrial causes of vaginal bleeding. Endometrial carcinoma with myometrial invasion is visible on magnetic resonance.⁸

The cervix also has a characteristic appearance on magnetic resonance. The isthmus is distinguishable as a dark region where the uterine contour is relatively

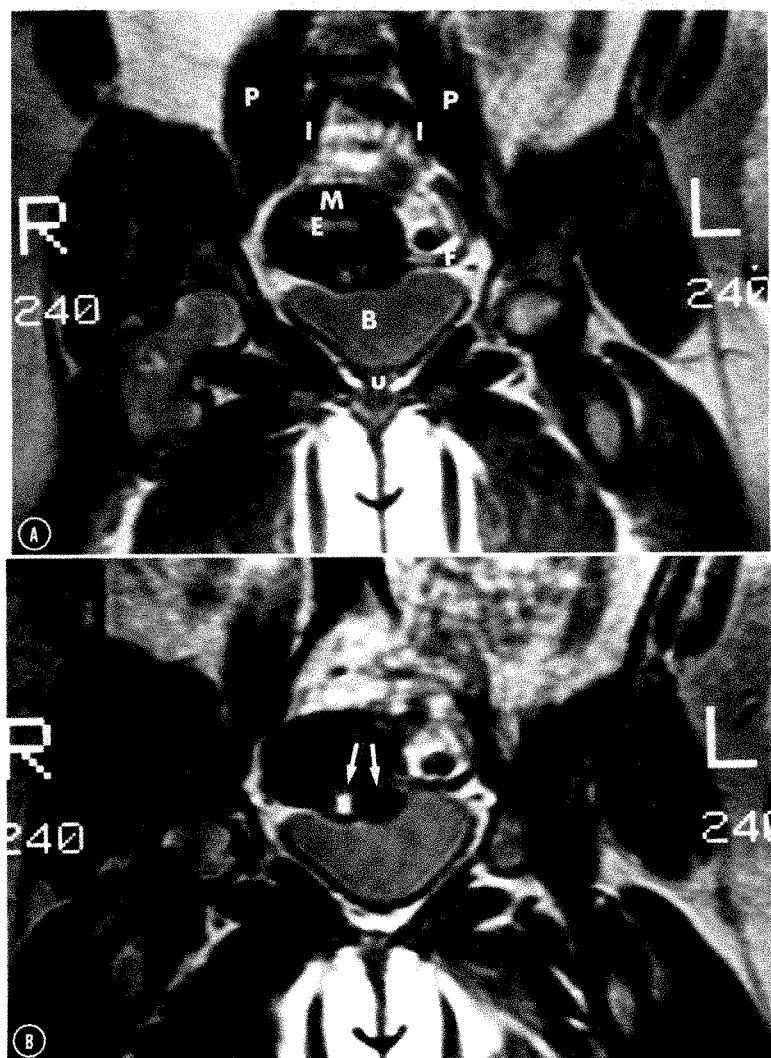


Fig. 3. A, Coronal image (1.5 T; SE TR 2000, TE 40) of pelvis demonstrating iliac (*I*) vessels, psoas (*P*) muscles, bladder (*B*) and its wall, urethra (*U*), fallopian tube (*F*), endometrium (*E*), and myometrium (*M*). Note the leiomyomas (*arrows*) better demonstrated on a more anterior section (**B**), which clearly demonstrates their varied signal intensity, bladder compression, and subserosal location (surgically confirmed). Ultrasound had suggested that these were ovarian masses.

constricted. Its intensity is lower than that of corpus smooth muscle because of the higher content of fibrous tissue. The external os is differentiated from the vaginal fornices and canal, which contain fluid and mucus. Since both the internal and the external os can be routinely defined in both nonpregnant and pregnant women, cervical length is readily measured.¹ The endocervical canal and glands are defined by a central high-intensity stripe, whereas the surrounding lower intensity area is the fibrous stroma. Such definition of cervical anatomy permits detection of early cervical cancer, as demonstrated in Fig. 4. Cervical pathology, particularly Stage I and II carcinoma, is not effectively diagnosed by computerized tomography or ultrasound.

In summary, the development of magnetic resonance

as an imaging tool is promising for the evaluation of gynecologic abnormality since the anatomy is so precisely demonstrated. Although it is currently more expensive and time-consuming than ultrasound or computerized tomography, there are several advantages. The unsurpassed contrast resolution allows appreciation of internal organ structure not seen by any other radiologic modality. Multiplanar views permit global visualization of disease along planes of spread. Finally, the technique is relatively safe, entailing no contrast material or ionizing radiation.

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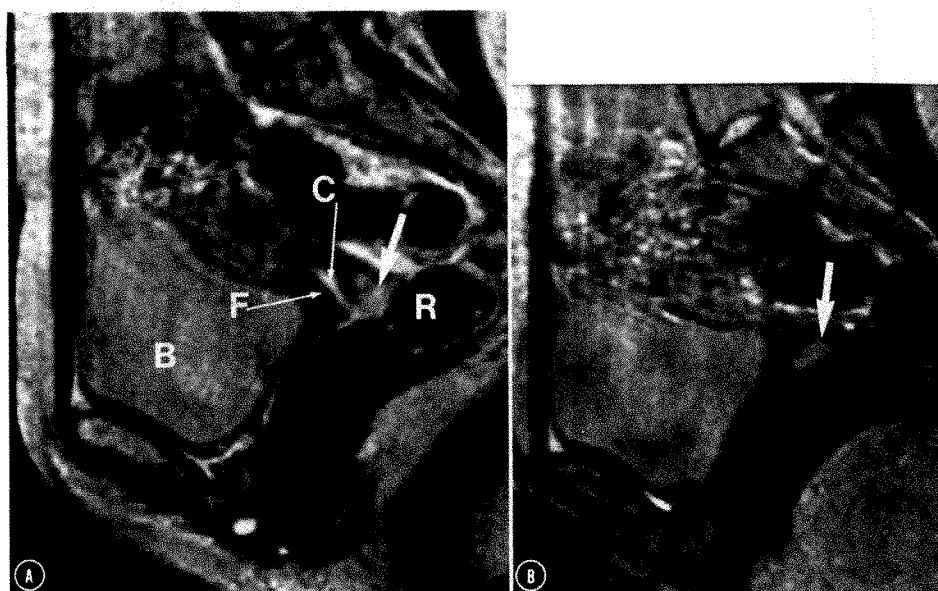


Fig. 4. **A**, Sagittal image (1.5 T; SE TR 2000, TE 40) demonstrating high signal area (large arrow) in posterior lip of cervix representative of a Stage II cervical cancer (surgically confirmed). Note that the posterior vaginal fornix is obliterated and that in **B**, a parasagittal section, the tumor (arrow) extends into the upper third of the vagina. *R*, Rectum; *B*, bladder; *C*, cervical canal; and *F*, fibrous stroma.

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Intrapartum treatment of preeclamptic hypertension by ketanserin

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Ketanserin was administered intravenously as either 20 or 10 mg boluses to 16 patients with severe preeclampsia in labor. The fetal heart rate was monitored with a scalp electrode and the uterine contractions were evaluated with a fluid-filled catheter connected to a physiologic pressure transducer. A 10-minute recording before every administration of ketanserin was compared to the same period immediately afterward. Ketanserin lowered the systolic and diastolic blood pressure significantly ($p < 0.005$, paired Student's *t* test). Maternal heart rate was unchanged but the mean fetal heart rate increased by 4.6 bpm. Long-term beat-to-beat variability and acceleration patterns were not affected. On four occasions an improvement in variable decelerations of the fetal heart, due to a reduction in uterine activity, was seen. Changes in the amplitude of contractions were not statistically significant. The frequency of contractions, however, was significantly reduced from 3.6 to 2.4 per 10 minutes ($p < 0.005$). No serious adverse effects occurred. (AM J OBSTET GYNECOL 1986;155:260-3.)

Key words: Ketanserin, preeclampsia, contractions, fetal heart rate

It is often necessary to control the blood pressure of patients with severe preeclampsia with drug therapy during the first stage of labor. Such a drug should be devoid of severe maternal side effects and should be safe to the fetus. Another prerequisite is that it should not interfere with the course of labor. Serotonin (5-hydroxytryptamine) has been shown to have an amplifying effect on platelet aggregation and it powerfully augments the constrictor responses to other neurohormonal transmitters, such as angiotensin II, histamine, and catecholamines. Aggregation of platelets releases serotonin, which in turn causes constriction of vascular smooth muscle.¹ As patients with preeclampsia are sensitive to the pressor response of angiotensin² and have reduced platelet counts,³ it is possible to postulate that serotonin plays a role in the modulation of the raised blood pressure in patients with preeclampsia. Ketanserin, a serotonergic antagonist that competitively and selectively blocks 5-hydroxytryptamine type 2 serotonergic receptors,⁴ may therefore be effective in the treatment of hypertension in patients with preeclampsia. Weiner et al.⁵ have recently demonstrated that ketanserin is effective in reducing blood pressure in puerperal patients with preeclampsia. The same group also administered ketanserin to a patient with a known intrauterine death and found an effective control of the blood pressure.⁶ In the light of these results, we decided

to study carefully the effect of ketanserin on maternal blood pressure, fetal heart rate, and uterine contractions during the first stage of labor.

Material and methods

Only patients with preeclampsia who were in the first stage of labor and in whom internal monitoring of the fetal heart rate and uterine contractions was possible, were included in the study. Sixteen patients were investigated. There were 10 primigravid patients, four patients with one previous pregnancy, and two with five previous pregnancies. Twelve patients received an initial bolus of 20 mg and the other four, an initial bolus of 10 mg. A total of 40 doses were given. Ketanserin doses varied between 10 and 90 mg. The mean gestational age was 37.5 weeks with an SD of 5.7 weeks. The mean amount of proteinuria present was 1 gm/L. The mean birth weight was 2734 gm with an SD of 783 gm.

Blood pressure was always taken while the patient was lying in the lateral position. The upper arm was used for all measurements. Phases I and IV (muffling) of the Korotkoff sound were used to identify the systolic and diastolic blood pressure, respectively. All measurements were done in the interval between contractions and the pulse rate was measured simultaneously. For the purpose of this study hypertension was defined as a blood pressure of $\geq 150/100$ mm Hg for four consecutive measurements taken at 15-minute intervals or a blood pressure of $160/110$ mm Hg for two consecutive measurements taken 15 minutes apart. To assess proteinuria, midstream urine specimens were tested with multiple reagent strips (Multistix, Ames).

Patients were excluded from the study for the fol-

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Table I. Effect of first-dose ketanserin on blood pressure, heart rate, and uterine contractions

Parameter	10 min before treatment		10 min after treatment		Wilcoxon signed rank test
	Mean	SD	Mean	SD	
Systolic blood pressure (mm Hg)	158.8	22.0	141.8	21.8	$p < 0.01$
Diastolic blood pressure (mm Hg)	114.6	10.8	97.5	16.1	$p < 0.005$
Maternal heart rate (bpm)	89.5	16.4	94.3	17.3	$p > 0.05$ (NS)
Fetal heart rate (bpm)	135.3	13.8	139.9	15.4	$p < 0.05$
Uterine contraction frequency per 10 min	3.6	1.2	2.4	0.8	$p < 0.005$
Basal tone (mm Hg)	7.5	5.9	7.4	5.3	$p > 0.05$ (NS)
Contraction amplitude (mm Hg)	42.5	18.9	42.4	19.7	$p > 0.05$ (NS)
Montevideo units	135.5	82.8	105.6	56.5	$p < 0.5$

Table II. Effect of ketanserin on blood pressure, heart rate, and uterine contractions with all doses considered

Parameter	Mean change	SE	Paired Student's <i>t</i> test	<i>p</i>
Systolic blood pressure (mm Hg)	14.1	3.9	3.68	<0.005
Diastolic blood pressure (mm Hg)	14.0	2.5	5.54	<0.0001
Maternal pulse rate (bpm)	-4.1	2.2	-1.86	>0.05 (NS)
Fetal heart rate (bpm)	-3.5	2.2	-1.60	>0.05 (NS)
Contraction frequency per 10 min	0.6	0.1	4.64	<0.0005
Basal uterine tone (mm Hg)	-0.3	0.3	-1.11	>0.05 (NS)
Contraction amplitude (mm Hg)	-1.9	2.7	-0.70	>0.05 (NS)
Montevideo units	19.8	10.3	1.92	>0.05 (NS)

lowing reasons: intrauterine death, fetal distress, any antihypertensive therapy in the preceding 12 hours, and epidural analgesia.

Uterine contractions were measured by means of a fluid-filled intra-amniotic catheter connected to the physiologic pressure transducer of a Hewlett-Packard cardiotocograph. The catheter was regularly flushed and calibrated. A paper speed of 1 cm/min was used throughout. At the end of the study, uterine contractions were assessed by examining the basal pressure, amplitude and frequency of contractions, and Montevideo units.

The fetal heart rate was monitored internally by means of a scalp electrode. The following heart rate patterns were assessed: accelerations, decelerations, basal heart rate, and long-term beat-to-beat variability. The exact moment of ketanserin administration was always indicated on the recording paper so that the period before the administration could be compared with the period immediately afterward. For the purpose of this study a 10-minute window immediately before the administration was compared with a 10-minute window immediately after the administration.

Ketanserin was administered as a 10 or 20 mg intravenous bolus. Supplementary boluses of 10 mg were administered every 10 minutes until the therapeutic objective of 140/90 mm Hg was obtained or when a maximum of 100 mg was administered. Ketanserin was usually discontinued once the patient had been deliv-

ered and other antihypertensive drugs were then used if the blood pressure remained $>160/110$ mm Hg. In our labor ward we use magnesium sulfate to prevent convulsions in patients with severe preeclampsia. Eight patients received a continuous infusion of this drug. Except for meperidine for pain and oxytocin, no drugs were used in these patients. The dosage of these drugs was not changed during the 20-minute period under observation.

For statistical analysis the paired Student *t* test and the Wilcoxon signed rank test were used. The amount of ketanserin administered depended on the length of labor as well as the response of the patient. As all patients did not receive equal amounts of ketanserin, only the first dose was considered for the first part of the analysis. For the second part all administrations were taken into account. The study was approved by the South African Drug Control Council and the Tygerberg Hospital Ethical Committee.

Results

The systolic blood pressures before treatment ranged between 220 and 130 mm Hg with a mean of 156.8 mm Hg. Diastolic blood pressure before treatment ranged between 100 and 140 mm Hg with a mean of 114.6 mm Hg. After administration of the first bolus dose of ketanserin, the systolic and diastolic values were 141.8 and 97.5 mm Hg, respectively (Table I). This decline in blood pressure was statistically significant.

No significant changes in the maternal pulse rate, basal uterine tone, and amplitude of contractions were observed. A significant increase in the fetal heart rate occurred ($p < 0.05$). The frequency of uterine contractions and the uterine contractility, as measured by Montevideo units, decreased significantly ($p < 0.5$). In some patients a reduction in the amplitude of contractions was noticed.

When the effects of all doses were considered, it was found that significant declines in both systolic and diastolic blood pressures were obtained, as well as a reduction in the frequency of uterine contractions ($p < 0.005$). Changes in the fetal and maternal heart rates, basal uterine tone, contraction amplitude, and Montevideo units were not statistically significant (Table II).

No change in the long-term fetal heart rate variability was observed. Ketanserin also had no effect on the acceleration patterns of the fetal heart rate. In four patients an improvement in variable decelerations of the fetal heart rate was observed. All these effects occurred when uterine activity was reduced. Administration of ketanserin caused no change in the beat-to-beat variability of the fetal heart rate in any patient. Acceleration patterns were present in 10 patients before the administration of ketanserin and no change was seen after the administration. The mean 5-minute Apgar score was 9 with an SD of 1.

Magnesium sulfate was administered before the ketanserin in eight patients. A reduction in the frequency of contractions was also seen in four patients who had not received magnesium sulfate. Three patients complained of dizziness and eight patients felt drowsy after administration of ketanserin. No other adverse effects were seen.

Comment

The study demonstrated that ketanserin effectively reduced the blood pressure in patients with preeclampsia during labor. It does not cause any change in the maternal heart rate. Except for dizziness and sleepiness no adverse maternal effects were seen.

The effect on the basal fetal heart rate is uncertain as a mean increase of 4.6 bpm was found when only the effects of the first administration of ketanserin were examined. When the effects of the 40 administrations were examined, the change in the basal fetal heart rate was not significant. No change in the long-term beat-to-beat variability of the fetal heart rate was seen. Acceleration patterns of the fetal heart rate were not affected by the administration of ketanserin. As an acceleration pattern of the fetal heart rate is usually indicative of fetal well-being, it is unlikely that ketanserin reduced fetal oxygenation. In fact, on four occasions an improvement in variable decelerations was

most likely due to a temporary reduction in frequency and amplitude of uterine contractions. Placental blood flow in patients with severe preeclampsia is reduced and therefore also perfusion of the intervillous space.⁸ Frequent and strong contractions could further reduce the perfusion of the intervillous space. As fetal oxygenation is dependent of the relaxation of the uterus between contractions, a reduction in the frequency of contractions may be beneficial to the fetus. This is exactly what happened with the administration of ketanserin.

The mean frequency of contractions was reduced from 3.6 to 2.4. As a frequency of 2.4 is in the normal range and as the amplitude of contractions did not change, it is unlikely that ketanserin could have an adverse effect on the progress of labor. The question arises whether the reduction of uterine contractility could not have been caused by magnesium sulfate administration, as it is well known that this drug reduces uterine activity.⁹ It is, however, unlikely because in four patients in whom no magnesium sulfate was administered, a reduction in uterine activity also occurred. In this study the 10-minute window before administration was compared to the period immediately afterward; therefore the possibility of other causes influencing the uterine activity is unlikely. At this stage it is uncertain in which way ketanserin reduces the frequency of contractions as serotonin receptors have not yet been described in the human uterus.

Although more information is needed to establish the safety of ketanserin during labor, the results of this study are promising as this drug has possible advantages, such as maternal sedation and reduction in contraction frequency, in the treatment of hypertension in patients with preeclampsia.

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Evidence of accelerated platelet production and consumption in nonthrombocytopenic preeclampsia

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Platelet size and the distribution of platelet sizes are both increased in preeclamptic patients with normal platelet counts. These changes suggest that accelerated platelet production and consumption are both widespread in preeclampsia. (*AM J OBSTET GYNECOL* 1986;155:263-5.)

Key words: Preeclampsia, platelets

Thrombocytopenia occurs in only a minority of preeclamptic patients. However, since increased marrow platelet production could mask increased consumption, the incidence of actual thrombocytopenia may not reflect the true incidence of platelet consumption in preeclampsia. The purpose of the study was to search for evidence of increased platelet production in nonthrombocytopenic preeclamptic patients. Since young platelets are larger, platelet size and the distribution of platelet size were evaluated in a selected group of preeclamptic patients.

Material and methods

A study group of 20 preeclamptic patients was selected from a larger group of 56 sequential preeclamptic patients for whom care was given at the Medical University of South Carolina. Thirty-six patients with one or more of the following were excluded: (1) any platelet count $<150,000/\text{mm}^3$, (2) labor, (3) abnormal bleeding, abruptio placentae, or disseminated intravascular coagulation, (4) any hematologic complications

or pregnancy such as abnormal hemoglobins or severe anemia, (5) earlier than third trimester. The second, third, and fourth exclusions are recognized or potential causes (such as stress) of platelet changes. Seven patients were severely preeclamptic. Severity of disease was not a criteria for selection. All preeclamptic patients had at least two platelet counts. The first platelet count and associated data were used for all preeclamptic patients.

The control group was composed of 21 patients having routine hematologic evaluation in preparation for elective repeat cesarean section. Except for not being preeclamptic, the control group patients met the same inclusion/exclusion criteria as did the preeclamptic patients.

Platelet count and platelet size (average platelet size = mean platelet volume) were determined by the Coulter S Plus 1 or S Plus 3 instruments. The normal range for mean platelet volume was 8.63 ± 0.87 fl in one study.¹ The distribution of platelet sizes is related to the platelet distribution width. The more scattered the pattern of platelet sizes in a specimen of blood, the larger the platelet distribution width. Mathematically, the platelet distribution width is $e^s \times 10$, where s is the standard deviation of the platelet size distribution and where e is the base of natural logarithms.

Group means were compared by Student's t test.

Results

The results are summarized in Table I. The mean platelet volume and platelet distribution width were

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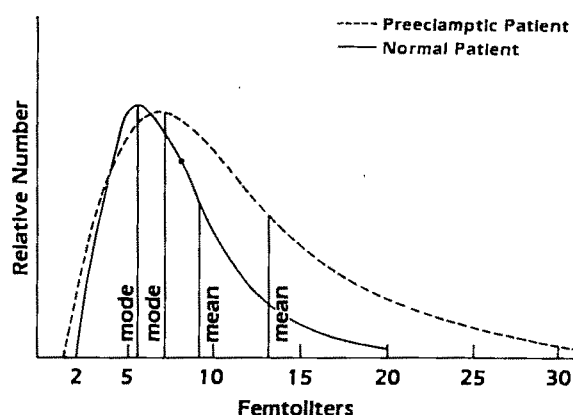


Fig. 1. Platelet size distribution plots for a preeclamptic patient (platelet count, 282,000/mm³; mean platelet volume, 13.3 fl; platelet distribution width, 18.5; modal size, 7.2 fl) and for a healthy obstetric outpatient (platelet count, 295,000/mm³; mean platelet volume, 9.2 fl; platelet distribution width, 16.0; modal size, 5.9 fl). The Y axes are relative numbers and are not directly comparable.

both significantly higher in the preeclamptic than in the control group, $p < 0.02$ for both comparisons. Notwithstanding the mean platelet volume and platelet distribution width evidence for large young platelets in the preeclamptic group, the mean platelet count in this preeclamptic group was actually lower than in the control group, $p < 0.02$.

Fig. 1 shows the distribution of platelet sizes in a preeclamptic patient in contrast to the distribution in a normal patient. Although the platelet counts are very similar, for the preeclamptic patient there is a widening of the distribution curve, a rise in the mean platelet volume and the modal (most frequent) size, and a "tail" of macrothrombocytes (very large platelets). The widening of the peak gives the preeclamptic patient a larger platelet distribution width. Statistically, the platelet distribution width is related to the variance ("scatter") of the platelet size distribution.

Comment

A proposed mechanism for thrombocytopenia in preeclampsia is platelet adherence to damaged vascular endothelium.² However, since only some preeclamptic patients become thrombocytopenic, this plausible theory also suggests that either (1) vascular endothelial damage is limited to only a fraction of preeclamptic patients or (2) vascular endothelial damage is more common but resulting platelet consumption is not detected by traditional means. The purpose of this study was to test the concept that in preeclampsia, increased platelet production can mask increased platelet consumption in nonthrombocytopenic patients.

Younger platelets are larger than others. Any condition that accelerates platelet turnover will result in an

Table I. Mean platelet size, platelet distribution width, and platelet count in preeclamptic and control patients

	Preeclamptic patients	Control subjects	<i>p</i>
Mean platelet volume (fl)	11.5 ± 0.4*	10.2 ± 0.3	<0.02
Platelet distribution width	17.4 ± 0.2	16.8 ± 0.1	<0.02
Platelet count/mm ³ × 10 ³	234 ± 13.6	287 ± 15.2	<0.02

*Mean ± SEM.

increase in new platelets in the peripheral circulation if the bone marrow is able to compensate.³ This will cause an increased mean platelet volume regardless of the platelet count. The increased platelet size (mean platelet volume) in the preeclamptic patients in this study strongly suggests that there is an unusually large population of young platelets present. Fay et al.¹ have previously shown the platelet distribution width to be elevated in preeclampsia. The increased platelet distribution width in the preeclamptic patients in this study reflects the widening (increased variance) of the distribution curve caused by the presence of an increased population of large, young platelets. Simultaneous, abnormal platelet consumption is suggested by the finding that the preeclamptic group had a lower mean platelet count in spite of the presence of a large outpouring of large young platelets. These data suggest that in preeclampsia, accelerated marrow platelet production is a widespread compensatory mechanism for accelerated platelet destruction by damaged vascular endothelium, even for preeclamptic patients with normal platelet counts.

The choice of 150,000/mm³ instead of 100,000/mm³ as a lower platelet limit serves two purposes in this study. First, it makes unlikely the chance that a patient could have a fluctuation in platelet count to below 100,000/mm³ and still be undetected by multiple samplings. Second, it tests the hypothesis of the study more strictly than would a lower limit of 100,000/mm³, since platelet abnormality is presumably less likely in a patient whose platelet count is <150,000/mm³ than in one whose count is only <100,000/mm³.

In this study, preeclamptic patients were selected by platelet count, not severity of disease. Giles and Inglis¹ measured platelet count and mean platelet volume and reached very similar conclusions in a study in which preeclamptic patients were grouped by severity of disease rather than by platelet count. The tail of platelet size distribution in the preeclamptic patients in this study probably represents the megathrombocytes (very large platelets) observed by Giles and Inglis.

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Incidence of preeclampsia among asthmatic patients lower with theophylline

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The incidence of preeclampsia was reduced among asthmatic patients taking theophylline (one of 85) compared to other asthmatic patients (six of 68, $p < 0.05$). Other factors did not appear to account for these results. Theophylline, which is known to inhibit platelet aggregation and alter vascular tone, may reduce the incidence of preeclampsia, at least among asthmatic patients. (*AM J OBSTET GYNECOL* 1986;155:265-7.)

Key words: Theophylline, preeclampsia, asthma

Although the etiology of preeclampsia remains unknown, platelet activation, the resultant release reaction products, and a generalized vascular spasticity appear to play an important role in its pathophysiology. Whether platelet activation is an inciting factor or the result of other stimuli such as increased vascular reactivity and secondary endothelial damage is controversial.

Some prophylactic and therapeutic regimens for preeclampsia have attempted to reduce platelet activation or modify the vascular effects of release reaction products. The combined administration of dipyridamole and low-dose aspirin has been used to reduce the incidence of preeclampsia in a high-risk group of patients. Phosphodiesterase inhibitors, for example, theophylline and dipyridamole, are inhibitors of platelet aggregation. Everett et al.¹ reported that administration of theophylline to patients with preeclampsia attenuated the pressor response to infused angiotension II. They suggested that theophylline reduced vascular reactivity by increasing levels of intracellular cyclic adenosine monophosphate (cAMP).

We hypothesized that the administration of theophylline would reduce the incidence of preeclampsia by inhibiting platelet activation and/or reducing vascular reactivity. As a preliminary evaluation of this hypothesis, we examined the effects of theophylline administration on the incidence of preeclampsia in a population of asthmatics.

Patients and methods

Data were collected from the records of 121 asthmatics delivering singleton live-born infants at our institution from 1982 to 1985. Additionally, 32 previous pregnancies of these patients were included in the study. The next singleton live-born delivery that was comparable for parity and with complete records available was used as one of 116 controls. Of the 153 pregnancies complicated by asthma, 85 were treated with theophylline for >4 weeks before delivery; 68 were not. For consistency, only patients with a discharge diagnosis of preeclampsia and receiving a standard protocol of treatment were considered preeclamptic in this study. The effects of administration of theophylline and sympathomimetics on the incidence of preeclampsia were analyzed, with asthma, maternal age, parity, smoking, and chronic hypertension considered as potentially confounding factors. Birth weight and gestational age were evaluated as secondary outcome measures. Fisher's exact probability test, χ^2 , and Student's t test were

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Table I. Incidence of preeclampsia among asthmatic patients according to medication and parity

Medication	Nulliparas		Multiparas		Total		<i>p</i>
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Theophylline	0/20	0	1/65	2	1/85	1	<0.05
No theophylline	3/18	17	3/50	6	6/68	9	
Sympathomimetics	1/21	5	3/70	4	4/91	4	NS
No sympathomimetics	2/17	12	1/45	2	3/62	5	

Table II. Clinical characteristics and secondary outcome measures among asthmatic and control subjects

Characteristic	Asthmatic		Controls		<i>p</i>
	<i>n</i>	%	<i>n</i>	%	
Age (yr)	25 ± 6.1		26 ± 5.6		NS
Nulliparity	38/153	25	23/116	20	NS
Smoking	71/153	46	32/116	28	<0.01
Chronic hypertension	17/153	11	5/116	4	<0.05
Gestational age (wk)	39 ± 2		39 ± 3		NS
Birth weight (gm)	3000 ± 680		3050 ± 650		NS

used for statistical analysis, with a two-tailed $p < 0.05$ considered significant.

Results

Among asthmatic patients, those treated with theophylline had a significantly lower incidence of preeclampsia (Table I). In contrast, no similar effect was demonstrated with sympathomimetics. The limited number of patients requiring steroids ($n = 13$) precluded meaningful statistical analysis.

Potentially confounding clinical characteristics and secondary outcomes are summarized in Table II. The incidence of preeclampsia did not appear to be influenced by asthma; seven of 153 asthmatic patients (5%) developed this complication compared to five of 116 control subjects (4%). Pregnancies complicated by asthma had higher frequencies of smoking and chronic hypertension than control pregnancies. No significant differences in the characteristics outlined in Table II were observed among asthmatic patients according to the type of medication administered (theophylline or sympathomimetics). Birth weight and gestational age did not appear to be significantly altered by asthma or its therapeutic regimens.

Comment

The primary finding of this study, a reduction of preeclampsia among asthmatic patients treated with theophylline, should be interpreted with caution in view of the limited number of patients with preeclampsia; a relatively small number of patients could have altered the results. However, achieving statistical significance at this sample size suggests a large effect of theophylline (for example, greater than the effect of parity in this study). Closer medical supervision could

also potentially modify the incidence of preeclampsia among asthmatic patients requiring medication; however, this explanation alone appears unsatisfactory, since asthmatic patients treated with only sympathomimetics did not have a similarly decreased incidence of preeclampsia. In the absence of any demonstrable effect of other potentially confounding factors, theophylline itself may be responsible for the reduced incidence of preeclampsia. This would corroborate previous studies reporting reductions of the frequency and severity of preeclampsia with the use of antiplatelet regimens.

On a theoretical basis, there are several reasons to believe that theophylline might reduce the incidence of preeclampsia. Prostacyclin, which is decreased in preeclampsia, inhibits platelet aggregation; therapeutic levels of theophylline potentiate these inhibitory effects.² At greater concentrations, theophylline inhibits synthesis of thromboxane, a platelet aggregator and vasoconstrictor.² Vasodilation via increasing levels of cAMP may be another mechanism by which theophylline could reduce the incidence of preeclampsia.

Asthmatic patients may not be representative of the general population with respect to theophylline administration and preeclampsia. However, the findings of this study deserve further consideration, since preeclampsia remains a significant source of morbidity and mortality. Confirmation is needed to determine whether theophylline administration is warranted among patients at risk for preeclampsia.

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Evaluation by computerized axial tomography of eclamptic women with seizures refractory to magnesium sulfate therapy

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Over a 1-year period, seven eclamptic patients with repetitive seizures while receiving therapeutic levels of intravenous magnesium sulfate were evaluated by computerized axial tomography. In five patients brain abnormalities were identified, which included diffuse cerebral edema (one), cerebral venous thrombosis (two), and low density white matter (two). Clinical management of eclamptic women on the basis of structural central nervous system abnormalities documented by computerized tomography appears to warrant further investigation. (*AM J OBSTET GYNECOL* 1986;155:267-8.)

Key words: Computerized tomographic scan, eclampsia, seizures, magnesium sulfate

Eclampsia is characterized by the presence of seizure activity in a pregnant patient without a preexisting history of neurological disease. Despite the increased use of computerized axial tomography in clinical medicine, it has rarely been employed in a systematic fashion to study central nervous system changes associated with eclampsia. This review was undertaken to determine whether or not structural central nervous system abnormalities could be identified by computerized tomographic scanning in eclamptic women with repetitive seizures despite therapeutic levels of magnesium sulfate.

Material and methods

From the period beginning May 1, 1984, to April 30, 1985, there were 15 eclamptic patients managed at Jefferson Davis Hospital in Houston, Texas. Seven patients were identified with persistent seizures despite therapeutic serum levels of magnesium sulfate (4 to 7 mEq/L). All seven patients had postpartum computerized tomographic scans of the head performed between 12 and 96 hours after their initial seizure episode. They received a standard regimen of intravenous magnesium sulfate (4 gm) over 20 minutes followed by a continuous infusion of 2 to 3 gm/hr. Treatment of these

repetitive seizures occasionally required other anti-epileptic medications such as phenytoin, phenobarbital, or diazepam. Mean blood pressures >126 mm Hg were controlled with either intravenous hydralazine, nitroglycerin, or nitroprusside by continuous infusion. Although a trial of labor was attempted in all seven patients, a cesarean section was ultimately required for obstetric indications in each case. There were no maternal deaths in this group. Mean values are reported with standard deviations.

Results

Seven eclamptic women with repetitive seizures were reviewed for clinical presentation and brain abnormalities detected by computerized tomographic scans. The mean age of this nulliparous group was 18.4 ± 3.3 years. Eclampsia was diagnosed at a mean gestational age of 35.7 ± 3.6 weeks. The presenting mean arterial blood pressure was 117 ± 5 mm Hg. Six of seven patients experienced repetitive seizures during the antepartum period. The mean serum magnesium ion level at the time of the first repetitive seizure was 5.8 ± 1.3 mEq/liter. Mean serum colloid osmotic pressure at admission was 22.2 ± 1.7 mm Hg. Past histories were unremarkable for chronic hypertension or seizure disorders. Brain abnormalities detected by computerized tomography in five patients included diffuse cerebral edema (one), cerebral venous thrombosis (two), and low-density white matter (two).

Table 1 correlates clinical presentation with these structural brain abnormalities. Four patients (2,3,5, and 6) presented with the prodromal symptoms of a wors-

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Table I. Correlation between computerized axial tomographic findings and neurological presentation in seven eclamptic women with persistent seizures receiving magnesium sulfate therapy

Patient No.	Computerized tomographic findings	Headache	Visual disturbances	Status
1	Cerebral edema	—	—	
2	Cerebral venous thrombosis	+	—	
3	Cerebral venous thrombosis	+	—	
4	Low-density white matter	—	+	
5	Low-density white matter	+	+	
6	Normal	+	—	
7	Normal	—	—	

ening headache over the 2 to 3 days before admission. Visual disturbances were noted in patients 4 and 5. Two women (3 and 5) developed status epilepticus and required ventilatory support.

Comment

Several authors have presented case reports of one to two eclamptic patients with unusual neurological manifestations by computerized tomography. Three of these reports included computerized tomographic evidence of diffuse cerebral edema, and all such patients were treated with fluid restriction, dexamethasone, and/or mannitol with subsequent resolution of symptoms. On this basis, one group has recommended routine postpartum computerized tomographic scans of the head on all eclamptic women to guide patient management. We also found that cerebral edema rapidly resolved in response to mannitol and fluid restriction. However, we do not presently advocate routine postpartum computerized tomographic scans to identify cerebral edema in the absence of unusual neurological manifestations in eclampsia.

There have been two reports describing the occurrence of low-density white matter in eclamptic patients. It is interesting that the tomographic appearance of low-density white matter in eclampsia is similar to the changes described for hypertensive encephalopathy in nonpregnant patients.¹ These low-density areas appear to represent focal collections of edema fluid which extravasate through ischemic segments of vessel wall. The only patients (4 and 5) who experienced prodromal visual symptoms before seizure activity had evidence of low-density white matter localized to the visual cortex (occipital region). By contrast, headache appeared to be a nonspecific finding in this series of eclamptic women.

Cerebral venous thrombosis has typically been associated with intense and progressively worsening headache, presumably related to inflammation of pain-sensitive veins and dura. Propagation of the clot into the superior sagittal sinus may cause recurrent seizures or increased cerebral edema. Emergent neurosurgical

intervention may be required if these changes progress to cerebral infarction. Anticoagulants and fibrinolytics are thought to be beneficial for this condition in a carefully selected patient.

Dehydration and hyperviscosity have been implicated in the development of cerebral venous thrombosis. The two patients (1 and 2) identified in this study were noted to have serum osmolalities of 24.3 and 23.0 mm Hg, respectively. These values appear to be consistent with severe dehydration as confirmed by a low pulmonary artery wedge pressure in one patient. These patients represented the highest serum colloid osmotic pressure measured in our series. This information suggests an assessment of volume status by measurement of pulmonary artery catheterization or serum osmolality. Motility pressure measurement may be helpful in identifying eclamptic women at risk for cerebral venous thrombosis.

Our findings are not consistent with those of Sibai et al.² who documented normal computerized tomographic scans of the head from 20 eclamptic patients with neurological signs or symptoms while receiving adequate doses of intravenous magnesium sulfate. These investigators concluded that computerized tomography is rarely indicated in the management of eclamptic patients.

These preliminary observations suggest that eclamptic seizures refractory to magnesium sulfate therapy are associated with structural central nervous system abnormalities. On this basis we recommend computerized brain tomography in the clinical situation.

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A complete list of references will be provided on request.

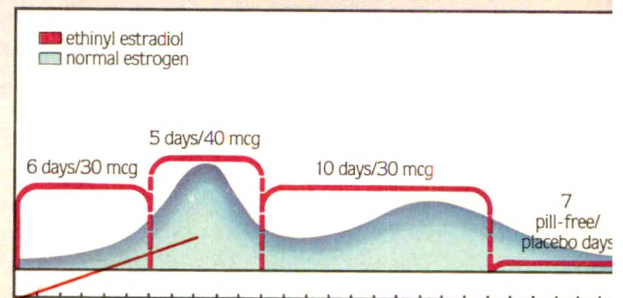
The image shows the front cover of a book. The cover has a dark blue, textured background. In the center, there is a rectangular area with a deep red, textured background. This red area is framed by a thin, gold-colored border. The title 'THE TEXTBOOK TRIPHASIC' is printed in a large, gold-colored, serif font, centered within the red area. Below the title, the text '1986 EDITION' is printed in a smaller, gold-colored, serif font. The overall design is classic and academic.

THE
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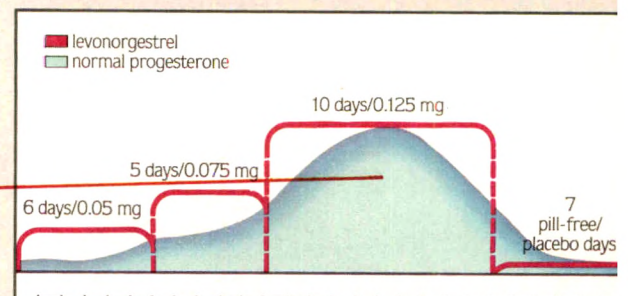
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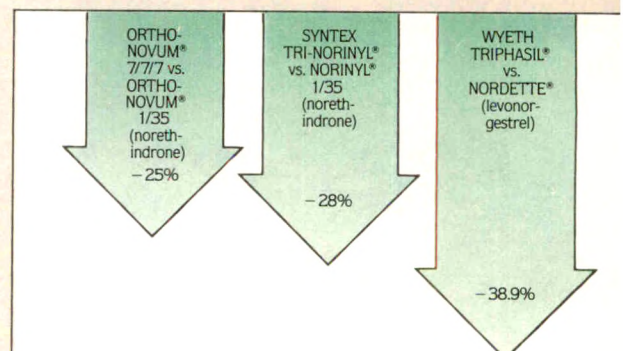
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VERMEULEN ²	6	↔	↔	↔	↔
HARVENGT ³	6	↔	↔	↔	↔
SKOUBY ⁴	6	↔	↑	↔	↔
MÄRZ ⁵	3	↔	↑	↔	↔
BRIGGS ⁶	18	↔	↑	↔	↔
CULLBERG ⁷	6	↔	↔	↔	↔
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TRIPHASIL[®] offers easy-to-remember Day 1 start, with no need for back-up contraception during the first cycle when taken as directed.[†] The discreet, reusable compa—preferred by patients and physicians¹¹—helps patients stay on schedule, month after month.

*Serious as well as minor adverse reactions have been reported following the use of oral contraceptives.

†No first-cycle pregnancies in 3,546 women using no additional protection. In worldwide postmarketing experience, only one first cycle pregnancy has been reported in the literature. (Graham H: Letter to Editor, *Br Med J* 284:422, 1982.) For dosage and administration, see brief summary on last page.



only **Triphasil**[®] Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen 
21- and 28-day regimens **A study in low-dose performance**

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Performance proven
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See important information on last page.

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IN BRIEF:

TRIPHASIL®—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage.—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications.—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

- Thromboembolic Disorders and Other Vascular Problems.**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.
- CEREBROVASCULAR DISORDERS.**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9 times greater in users than in nonusers.
- MYOCARDIAL INFARCTION (MI).**—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.
- RISK OF DOSE.**—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.
- ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES.**—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women. In those with long duration of use and in cigarette smokers it was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.
- PERSISTENCE OF RISK OF VASCULAR DISORDERS.**—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.
- Ocular Lesions.**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
- Carcinoma.**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.
- Hepatic Tumors.**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.
- Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring.**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4- to 7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence

from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly types of polyloidy are increased among abortions from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

Gallbladder Disease.—Studies report increased risk of surgically confirmed gallbladder disease in users of OC and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In the other studies, increased risk was apparent between 6 and 12 months' use.

Carbohydrate and Lipid Metabolic Effects.—Decreased glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

Elevated Blood Pressure.—Increase in blood pressure has been reported in patients on OC's. In some women hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

Headache.—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

Bleeding Irregularities.—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional cause should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

Ectopic Pregnancy.—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

Breast-feeding.—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until a breast has been weaned.

Precautions—GENERAL.—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorder, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's jaundice develops. OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing (a) Increased sulthobromophthalen retention

(b) Increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin III; increased norepinephrine-induced platelet aggregability.

(c) Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measure protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

(d) Decreased pregnandiol excretion

(e) Reduced response to metyrapone test.

Information for the Patient.—See Patient Package Labeling.

Drug Interactions.—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenylbutazone, ampicillin and tetracycline.

Carcinogenesis.—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings

Nursing Mothers.—See Warnings.

Adverse Reactions.—An increased risk of these serious adverse reactions has been associated with use of OC's. Warnings: thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Na and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation following immediate postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steep intolerance) to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, catarracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose.—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration.—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

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Missing hospital records: A confounding variable in retrospective studies

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In retrospective chart reviews there are often a certain number of missing hospital records. To elucidate this variable we compared the outcomes of very low birth weight breech infants with respect to the method of collecting data. A prospective sampling, during the hospital stay, of data was performed in 1979 to 1980 and in 1983 to 1984, and the frequencies of very low birth weight were 1.89 and 1.90, respectively, per 1000 live births. For 1981 to 1982 a retrospective record search was performed with the use of the ordinary medical record search system at this institution. For this period 39 of 52 (75%) hospital records were recovered, giving an apparent frequency of 1.32/1000 live births, which differed significantly from either period studied prospectively. An analysis of demographic data of the three groups revealed that the mean gestational age and the mean birth weights were higher in the period studied retrospectively compared with both periods studied prospectively and that the neonatal mortality rates were higher in the periods studied prospectively (74.1% and 57.1%, respectively) than in the period studied retrospectively (28.2%). It is concluded that the more complicated a clinical case is, the more likely the record will not be found for retrospective chart review. This problem should be kept in mind, and it ought to be a requirement that the number of missing charts be stated in retrospective observational studies. (AM J OBSTET GYNECOL 1986;155:269-71.)

Key words: Breech delivery, cesarean section, retrospective study, research design

In a climate of increasing hospital costs and decreasing available research funds the use of observational, nonexperimental retrospective types of studies must be considered. Such studies have provided important data in a number of clinical areas, but because of their inherent limitations they have also led to conflicting and contradictory conclusions.

Several methodologic deficiencies, such as patient selection, nonrandom allocation, and interpretation bias, can distort or invalidate retrospective observational studies.¹⁻⁶ On the other hand, observational studies do have their advantages. They are inexpensive, require no clinical intervention, require few ethical considerations, and make it possible to obtain large numbers of cases in a relatively short time.

The sampling process used in obtaining the data may have an occult bias and therefore may introduce misleading information. In retrospective chart reviews

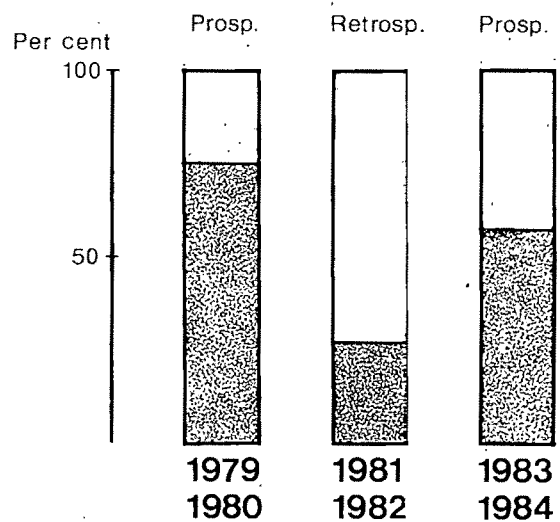


Fig. 1. Neonatal mortality in the three studied periods.

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there often are hospital records that are unavailable for review. Authors of such studies often make the assumptions that the patients with missing hospital records are randomly distributed with respect to the studied variable and that one can replace the missing information by increasing the sample size. To test this hypothesis we compared the outcomes of very low birth weight breech deliveries with respect to the method of collecting the data.

Table I. Demographic data from the three study periods

	<i>Prospective, 1979-1980</i>	<i>Retrospective, 1981-1982</i>	<i>Prospective, 1983-1984</i>
Total no. of deliveries	26,510	29,583	33,215
No. of singleton VLBW breech deliveries	54	39	63
Rate per 1000 live births	1.89*	1.32	1.90*
Characteristics			
Maternal age (mean $\pm$ SD) (yr)	22.8 $\pm$ 5.0	24.3 $\pm$ 5.1	23.6 $\pm$ 2.6
Parity (mean $\pm$ SD)	2.0 $\pm$ 1.5	2.6 $\pm$ 1.2	1.9 $\pm$ 1.6
Gestational age† (mean $\pm$ SD) (wk)	32.5 $\pm$ 4.6‡	34.7 $\pm$ 4.3	31.9 $\pm$ 4.2‡
Birth weight (mean $\pm$ SD) (gm)	1240 $\pm$ 370‡	1410 $\pm$ 400	1190 $\pm$ 395‡
Neonatal mortality	40/54 (74.1%)§	11/39 (28.2%)	36/63 (57.1%)

VLBW = Very low birth weight. All unsymboled values were not significantly different between groups.

* $p < 0.001$ for prospective period versus retrospective period.

†Estimated from Dubowitz score.

‡ $p < 0.05$ for prospective period versus retrospective period.

§ $p < 0.001$ for prospective period versus retrospective period.

|| $p < 0.1$ for prospective period versus retrospective period.

Material and methods

Patients giving birth to a singleton infant in breech presentation weighing between 750 and 1500 gm at the Los Angeles County/University of Southern California Medical Center between 1979 and 1984 were studied. During the period involved, a prospective study of the data was performed in 1979 to 1980 and 1983 to 1984. Data were then collected from the patient's chart while the patient remained in the hospital and were transcribed onto a worksheet for subsequent analysis. For 1981 to 1982 no prospective continuous data collecting was performed and for this time period a retrospective record search was done with the use of the ordinary medical record search system at this institution. Each chart goes through the following steps: ward, central discharge unit, utilization review, deficiency review, doctor's study, and file room. The chart is tracked with a central card that identifies the station where the chart is currently located. If a chart is requested, the central system is used to determine the location of the chart. In the present study the charts were requested twice according to the above-described system.

The two prospective collection periods (1979 to 1980 and 1983 to 1984) were compared with the retrospectively studied period, 1981 to 1982. For statistical analysis Student's t test and χ^2 were applied. The p values above 0.05 were not considered to be significant.

Results

Very low birth weight breech deliveries occurred in the same frequency in the two prospective study periods (1979 to 1980 and 1983 to 1984): 1.89 and 1.90 per 1000, respectively.

For the years 1981 to 1982, 39 of 52 (75%) hospital records were recovered (retrospective study). In the retrospectively studied period (1981 to 1982), the observed frequency was significantly lower than in either

prospectively studied period, 1.32/1000 live births ($p < 0.001$ for each). An analysis of demographic data of the three groups did not reveal any significant differences between the two prospectively studied periods, whereas the mean birth weight and the mean gestational age were higher in the retrospectively studied period compared with the early and late prospectively studied periods ($p < 0.05$ for 1979 to 1980, $p < 0.05$ for 1983 to 1984, respectively) (see Table I).

Fig. 1 shows the neonatal mortality (0 to 28 days) in the three studied periods. The neonatal mortality rates were higher in the prospectively studied periods (74.1% and 57.1%) than in the retrospectively studied period (28.2%; $\chi^2 = 19.3$, $p < 0.001$, for 1979 to 1980; $\chi^2 = 8.1$, $p < 0.01$, for 1983 to 1984).

Comment

The present study reveals a lower neonatal mortality in very low birth weight breech infants born in 1981 to 1982 compared with the same category of infants born in 1979 to 1980 and 1983 to 1984. A selection bias caused by an inability to locate missing hospital charts in the retrospectively studied period (1981 to 1982) is the most likely explanation for this difference since the obstetric management of these patients did not differ⁷ and our neonatal intensive care unit service reported a steadily improving survival rate year by year of all very low birth weight infants during the period involved (Teberg A, personal communication). Moreover, the demographic data show that the mean birth weight and gestational age were higher in the retrospectively studied period, suggesting that the missing charts (25%) probably included a larger proportion of infants with low birth weight and gestational age. Thus the more complicated a case is, the more likely the record will not be included in a retrospective chart review.

Our 25% rate of missing patient charts is high, but unfortunately high rates have been observed in other retrospective chart reviews at this institution. The location of the missing hospital records can only be speculated on; they may have been misfiled, removed for zealous investigation, or requested administratively because of recognized adverse outcome; finally, the possibility of removal for the purpose of limiting medicolegal exposure or promoting claims must be considered.

Although some patient charts are missing in most clinical settings, rather few authors mention this problem in retrospective studies. This problem will not be overcome by enlarging the sample size of the retrospective study. The only way to manage this problem is to reduce the number of missing charts to a minimum, and it ought to be a requirement that the number of missing charts be given in retrospective observational studies.

The recent introduction of computerized data bases makes it seem that the problem of missing records can

be solved. However, the use of such a data base will not guarantee a perfect solution to this problem because of difficulties inherent in chart coding, data entry, data manipulation, and misinterpretation. Nevertheless, a carefully executed retrospective study can provide important clinical information.

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Assessment of menstrual cycle symptoms by trend analysis

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The physical, psychological, and behavioral changes associated with the menstrual cycle can be assessed statistically by time series analysis. One such method, Trigg's technique for trends, has been adapted for the study of prospective symptom ratings used in evaluation of the premenstrual syndrome. Such analysis provides both qualitative and quantitative information concerning menstrual cycle symptomatology. The pattern of symptoms, as denoted by Trigg's tracking signal, can be identified. The premenstrual syndrome can be defined mathematically in terms of significant symptom trends at specified times in the menstrual cycle. The overall severity of symptoms at any point in the cycle can be gauged by the exponentially smoothed average symptom ratings. A derived statistic, the menstrual cycle ratio, is proposed as a global index of menstrual cycle morbidity which can be easily standardized to allow for comparability of research reports. (*AM J OBSTET GYNECOL* 1986;155:271-7.)

Key words: Menstrual cycle, prospective symptom ratings, trend analysis

Premenstrual syndrome is a common complaint, affecting an estimated 20% to 40% of women during their reproductive years.¹ Although there is much controversy concerning etiology and treatment, arguably the major confusion arises from a lack of a universally

agreed on definition and diagnostic criteria.² In the absence of a biochemical marker for the condition it is generally believed that prospective daily symptom rating is the most sensitive method of distinguishing non-cyclical problems from those that are essentially only present before menstruation.²

The evaluation of these subjective psychological, behavioral, and physical changes is difficult. Numerous methods have been suggested in the literature, ranging from simply the presence or absence of certain common complaints³ to the grading of the severity of symptoms by means of scores or linear analogue scales.⁴ Numer-

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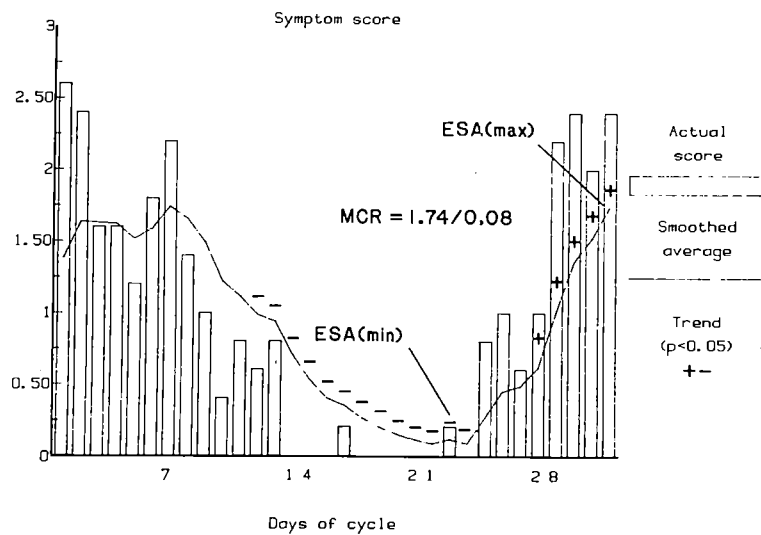


Fig. 1. Trend analysis of symptoms during a menstrual cycle showing significant trends that fulfill the criteria for the premenstrual syndrome. Symptoms scored on a scale of 0 to 3 (no symptoms to severe symptoms). Smoothing constant = 0.25. ESA(max) = Maximum exponentially smoothed average. ESA(min) = Minimum exponentially smoothed average. MCR = Menstrual cycle ratio.

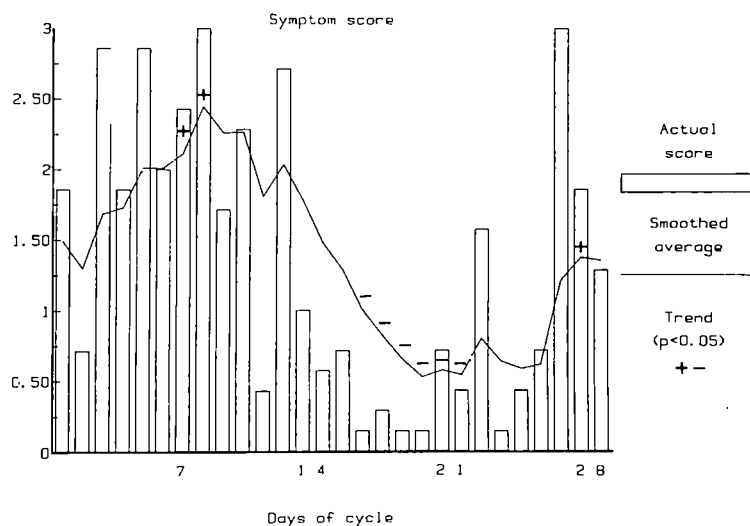


Fig. 2. Trend analysis of symptoms during a menstrual cycle showing significant trends that do not fulfill the criteria for the premenstrual syndrome. Symptoms scored on a scale of 0 to 3 (no symptoms to severe symptoms). Smoothing constant = 0.25.

ical methods allow for statistical analysis of the measurements, and simple but arbitrary formulas have been proposed,⁵ as well as adaptation of more complex mathematical models.⁶ The suitability of all these methods for the assessment of menstrual cycle data and for the detection of nonrandom events is uncertain.

The specific statistical approach to chronologic data, that is, observations made sequentially in time, is time series analysis. The special features of this method are, first, that the time order of the observations is important; second, that the observations need not be independent of each other; and third, that when successive

observations are dependent, future values can be predicted with a variable degree of accuracy.⁷ Daily symptom ratings appear to be ideally suited to such analysis, since the data are chronologic and dependent (that is, a "bad" day is more likely to be followed by another bad day and vice versa). Several techniques are available depending on the objectives of the analysis. One method of trend analysis, originally described by Brown⁸ and later modified by Trigg⁹ for the detection of long-term changes in the mean and widely used in industrial process control, has already been adapted to monitoring physiologic and pharmacologic responses in

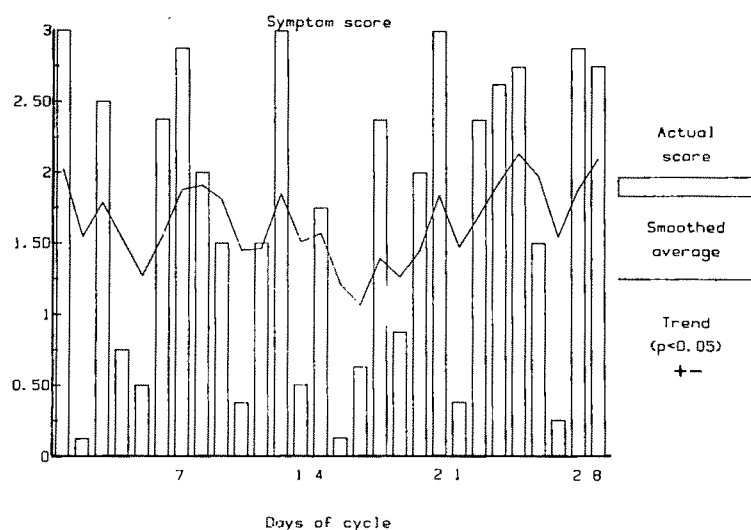


Fig. 3. Trend analysis of symptoms during a menstrual cycle showing absence of significant trends. Symptoms scored on a scale of 0 to 3 (no symptoms to severe symptoms). Smoothing constant = 0.25.

intensive care¹⁰ and to the control of analytical measurements in clinical chemistry.¹¹ More recently, and pertinent to the study of menstrual cycle symptomatology, Forrest¹² used a modified form of Trigg's technique in a study of the cyclical mood changes associated with the combined contraceptive pill.

As the first part of a study into the symptom characteristics of women complaining of premenstrual syndrome, we would like to present the theoretic basis of Trigg's technique for trends and its application in diagnosis and the assessment of menstrually related symptoms.

Description of Trigg's technique for trends

For a particular time series of observations

$$x(1), x(2), x(3), x(4), \dots, x(n)$$

Trigg's trend analysis⁹ uses the exponentially smoothed average, $ESA(n)$, as predictor of the next observation, $x(n+1)$. Exponential smoothing involves taking geometrically weighted sums of the past observations so that greatest weight is placed on the most recent observation

$$ESA(n) = sc \cdot x(n) + sc(1 - sc) \cdot x(n-1) + sc(1 - sc)^2 \cdot x(n-2) + \dots$$

where the smoothing constant, sc , has a range of 0 to 1. The above calculation can be simplified to

$$ESA(n) = sc \cdot x(n) + (1 - sc) \cdot ESA(n-1)$$

Successive observations allow for the calculation of the forecast error, $FE(n)$, that is the difference between the predicted and actual observations:

$$FE(n) = x(n) - ESA(n-1)$$

Trigg's technique uses the exponentially smoothed forecast error, $SFE(n)$, as an index of the degree of change in the observations

$$SFE(n) = sc \cdot FE(n) + (1 - sc) \cdot SFE(n-1)$$

and the exponentially smoothed absolute forecast error, called the mean absolute deviation, $MAD(n)$, as an index of the degree of random variation in the data

$$MAD(n) = sc \cdot |FE(n)| + (1 - sc) \cdot MAD(n-1)$$

Trigg's tracking signal, TS , is then defined as the ratio

$$TS(n) = SFE(n)/MAD(n)$$

When observations are relatively constant, the expected and observed values are similar, and both the forecast errors and the tracking signals will fluctuate around zero. With random observations all parameters will also fluctuate randomly above and below zero. However, when the observations consistently change in one direction, the smoothed forecast errors will become increasingly negative (observations becoming smaller) or positive (observations becoming larger), and the tracking signal will approach the theoretical minimum (-1) or maximum ($+1$), respectively.

At these two extremes of the tracking signal, it is certain that there has been a significant decrease or increase in the average value of the parameter measured. With tracking signals between the two extremes, the degree of significance of the tracking signal is a function of the smoothing constant. Batty¹³ showed that (1) the smoothed forecast errors are generally normally distributed irrespective of the distribution of the observations; (2) the local estimate of the mean absolute deviation is both relatively constant and approximates to the true mean absolute deviation, at least when the

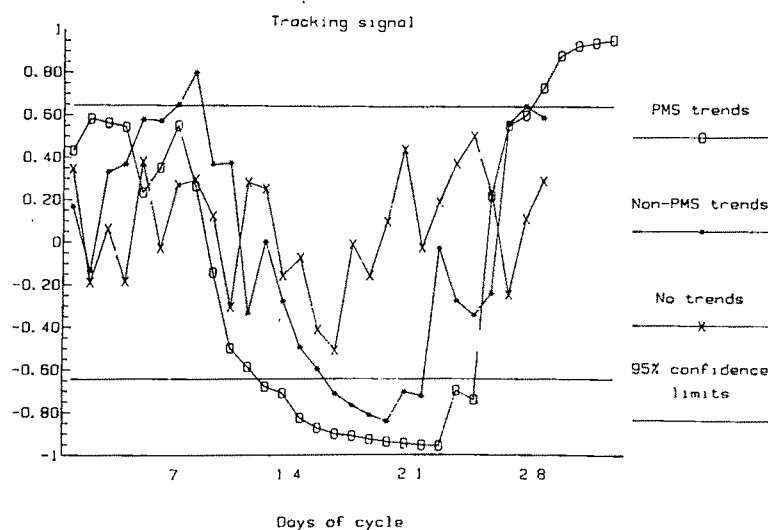


Fig. 4. Daily Trigg's tracking signals during a menstrual cycle for the different symptom trends. Smoothing constant = 0.25.

smoothing constant is small ($sc < 0.1$); and (3) the tracking signal is then also normally distributed about zero with a variance that is dependent on the variance of the smoothed forecast errors. Based on this relationship, Batty¹³ was able to calculate theoretic ($sc < 0.1$) and simulated ($sc > 0.1$) confidence limits for the tracking signal for various values of the smoothing constant.

Application of Trigg's technique for the assessment of symptoms

Prospective daily symptom ratings form a time series that can be analyzed both qualitatively and quantitatively by Trigg's technique for trends; any psychometric tool fulfilling these criteria can be analyzed, examples ranging from extensive symptom lists such as Moos' menstrual distress questionnaire¹¹ to simpler visual analogue scales dealing with major symptoms only.¹ The tracking signals provide information concerning the pattern of symptoms during the menstrual cycle and allow for the detection of statistically significant changes (that is, trends) in symptomatology in terms of timing, direction, and duration. Conversely the exponentially smoothed averages are an index of the overall magnitude of symptoms at any point in the menstrual cycle which take account of previous symptomatology. Such information can be utilized for the diagnosis of premenstrual syndrome and in the global assessment of the severity of symptoms.

Qualitative assessment of symptoms and diagnosis of premenstrual syndrome. Premenstrual syndrome can be considered as a condition associated with "distressing physical, psychological and behavioral symptoms, not caused by organic disease, which regularly recur during the same phase of each menstrual (ovar-

ian) cycle, and which significantly regress or disappear during the remainder of the cycle."¹²

Although such a definition is useful, it is subject to important ambiguities of interpretation. This is particularly so with reference to what is considered (1) distressing as well as (2) fluctuating in phase with the menstrual cycle. Whereas the severity of symptoms cannot be anything but subjective, making a quantitative threshold for the degree of "distress" not only inappropriate but of questionable practical value, Trigg's technique does allow for the objective evaluation of the timing of symptoms during the menstrual cycle by determining the pattern of statistically significant symptom trends. Thus if positive trends are equivalent to worsening symptoms (symptom scores increasing) and negative trends to improving symptoms (symptom scores decreasing), premenstrual syndrome could be defined as a condition associated during the menstrual cycle with (1) distress, (2) significant ($p < 0.05$) positive symptom trends at some time during the 14 days before menstruation and at no other time in the cycle, and (3) significant ($p < 0.05$) negative symptom trends at some time after the onset of menstruation and at no time after the presence of significant positive trends.

A typical example of this particular pattern of symptom trends is shown in Fig. 1. Alternative trend patterns include those in which significant symptom trends do not fulfill the above definition (for example, Fig. 2) and symptom ratings which do not result in any significant trends during the menstrual cycle (for example, Fig. 3). Trigg's tracking signals for the same data are plotted in Fig. 4, showing when in the cycle the upper and lower 95% confidence limits are exceeded, which gives rise to significant positive and negative trends, respectively.

Table I. Trend pattern of symptoms during a menstrual cycle with different smoothing constants

Day of cycle	Symptom rating*	Smoothing constant									
		0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
1	2.6										
2	2.4										
3	1.6										
4	1.6										
5	1.2										
6	1.8										
7	2.2	+	+	+							
8	1.4	+	+								
9	1.0	+									
10	0.4										
11	0.8										
12	0.6					-	-	-	-	-	-
13	0.8					-	-	-	-	-	-
14	0.0			-	-	-	-	-	-	-	-
15	0.0			-	-	-	-	-	-	-	-
16	0.0		-	-	-	-	-	-	-	-	-
17	0.2		-	-	-	-	-	-	-	-	-
18	0.0		-	-	-	-	-	-	-	-	-
19	0.0		-	-	-	-	-	-	-	-	-
20	0.0	-	-	-	-	-	-	-	-	-	-
21	0.0	-	-	-	-	-	-	-	-	-	-
22	0.0	-	-	-	-	-	-	-	-	-	-
23	0.2	-	-	-	-	-	-	-	-	-	-
24	0.0	-	-	-	-	-	-	-	-	-	-
25	0.8	-	-	-							
26	1.0	-	-				+	+	+	+	+
27	0.6	-	-				+	+	+	+	+
28	1.0	-				+	+	+	+	+	+
29	2.2				+	+	+	+	+	+	+
30	2.4				+	+	+	+	+	+	+
31	2.0			+	+	+	+	+	+	+	+
32	2.4		+	+	+	+	+	+	+	+	+

+, Significant positive trend. -, Significant negative trend.

*Symptoms scored on a scale of 0 to 3 (no symptoms to severe symptoms).

It is evident from the description of Trigg's technique that the important variable in the analysis is the smoothing constant, since it modulates the calculation of the smoothed averages and the tracking signals as well as the statistical interpretation of the latter. Exponential smoothing is analogous to the calculation of a moving average, and the smoothing constant is directly related to the number of observations (N) in an equivalent moving average*:

$$sc = 2/(N + 1)$$

From the above relationship it is apparent that a small smoothing constant is equivalent to a large number of observations in the moving average, while a large constant is conceptually similar to a small number of observations. Therefore, small or large smoothing constants in the analysis provide information about long-term and short-term trends, respectively. The choice of the smoothing constant depends on the properties of the time series and is commonly between 0.1 and 0.3.⁷ With assumption that an average menstrual cycle consists of approximately 28 consecutive ratings and

with consideration of typical plots of symptom scores one would expect in premenstrual syndrome (for example, Fig. 1), the use of a smoothing constant of 0.25, equivalent to a moving average of seven observations (days) is reasonable (Chatfield, personal communication). It is the smoothing constant that is typically associated with the maximum sum of smoothed errors squared ($\sum SE^2$) for this type of data and therefore can be considered the most sensitive to symptom changes. As can be seen from the trend analysis of the same set of data with use of various smoothing constants (Table I), the precise value of the constant is generally not critical. This is further highlighted if symptom trends, in this case during the first 7 days, are ignored to allow for equilibration of the exponentially smoothed averages during that time and thereby to reduce the chance of type II error in the analysis.

Quantitative assessment of symptoms—the menstrual cycle ratio. Each individual, exponentially smoothed average is a function of the most recent as well as the previous daily symptom ratings and so represents a global index of the severity of symptoms up

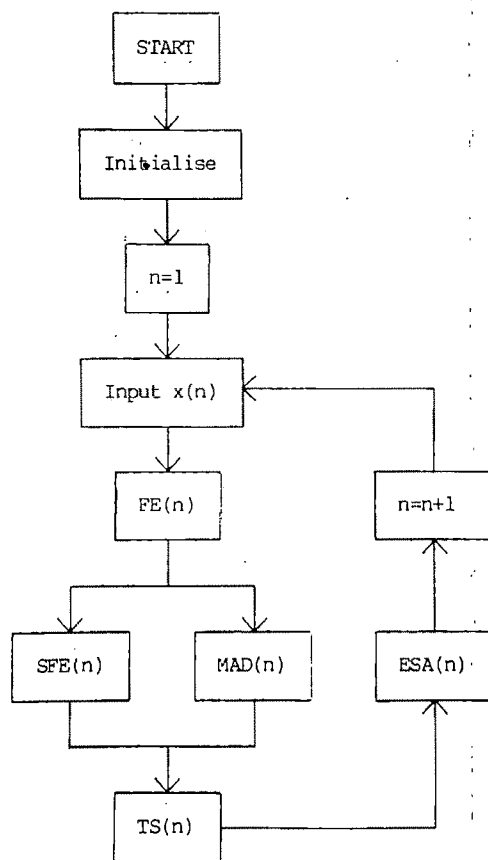


Fig. 5. Flow chart of Trigg's trend analysis for series of observations $x(1)$, $x(2)$, $x(3)$, ..., $x(i)$. FE = Forecast error. SFE = Smoothed forecast error. MAD = Mean absolute deviation. TS = Tracking signal. ESA = Exponentially smoothed average.

to that time in the menstrual cycle. This property of the smoothed averages can be utilized to describe the overall degree of complaining up to any specified point of a particular menstrual cycle (for example, midcycle or premenstrual day). Furthermore, the maximum, $ESA(\max)$, and minimum, $ESA(\min)$, exponentially smoothed averages within a cycle can be used in a menstrual cycle ratio (MCR):

$$MCR = ESA(\max)/ESA(\min)$$

where $ESA(\max)$ represents peak and $ESA(\min)$ baseline symptomatology. This ratio can be used as a quantitative index of menstrual cycle symptoms both for individuals and groups of patients. Thus, in classical premenstrual syndrome one would expect $ESA(\max)$ to be high and $ESA(\min)$ to be low, whereas with premenstrual exacerbation of chronic symptoms, $ESA(\min)$ would also be relatively high. As an example, the MCR for the daily symptoms depicted in Fig. 1 is:

$$MCR = 1.74/0.08$$

The MCR can also be standardized for different scoring

schemes and methods of assessment by expressing each element of the ratio as a proportion of the theoretic maximum daily score. Such a transformation gives a standardized menstrual cycle ratio (SMCR) which, provided identical smoothing constants are employed in the analysis (for example, 0.25), would allow comparisons to be made between research reports in terms of patient characteristics and response to therapy. In the example given in Fig. 1 above, where the symptoms were originally rated on a scoring of scheme of 0 to 3 (0, no symptom, to 3, severe symptoms), the MCR can be standardized by dividing both components by three, the theoretical maximum daily rating, giving:

$$SMCR = 0.58/0.03$$

Conclusions

Trend analysis appears to be a rational and valid statistical approach to the prospective assessment of the symptoms associated with the menstrual cycle. It can be used to (1) identify and classify symptom patterns, (2) provide a mathematical definition of premenstrual syndrome in terms of significant symptom trends at definable times in the cycle, (3) quantify symptoms, and (4) give a global index of menstrual cycle morbidity as expressed by the menstrual cycle ratio. While the complexity of the mathematics of the technique makes it unsuitable for use outside specialized clinics, trend analysis may have a wider role in gynecology for the study of other cyclical phenomena, such as endocrine and metabolic changes, for which daily measurements are possible.

We would like to thank Dr. A. R. W. Forrest for help with the implementation of a computer program to perform Trigg's trend analysis. A listing of the program in BBC BASIC suitable for the BBC-B computer running Z80 CP/M is available from us.

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Appendix

A flow chart for Trigg's technique for trends is shown in Fig. 5. At the start of the analysis of a series of data

by Trigg's technique for trends, certain parameters have to be initialized. For the purposes of this study investigating daily symptoms ratings during a menstrual cycle, the following initial settings were used:

Smoothing constant	sc	0.25
Smoothed forecast error	SFE(0)	0.0
Mean absolute deviation	MAD(0)	$\text{SQR}([2/\pi] \cdot [\text{Series SD}])$
Exponentially smoothed average	ESA(0)	Series mean

For each member of the time series, the forecast error (FE), smoothed forecast error (SFE), tracking signal (TS) and significance level are calculated. The above variables are updated together with the exponentially smoothed average (ESA) before analysis of the next member of the sequence.

Trend analysis of the symptoms of 150 women with a history of the premenstrual syndrome

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The daily symptom records of 150 untreated women with a convincing history of the premenstrual syndrome were investigated qualitatively and quantitatively by means of time series analysis in the form of Trigg's technique for trends. Symptoms were monitored with use of a modified menstrual distress questionnaire. Analysis showed that, depending on the symptom cluster, 60.7% to 85.3% of the women showed symptom trends consistent with the syndrome, 14.0% to 35.3% had trends not typical of the syndrome, and in 0% to 5.3% of records significant trends were absent. Since over 80% of the women were found to have premenstrual syndrome trends for three or more of the six symptom clusters studied, including 32% who showed this trend pattern for all symptoms, a retrospective history of premenstrual syndrome is likely to be confirmed on prospective assessment for at least some symptoms. Quantitatively women who fulfilled the diagnostic criteria for premenstrual syndrome differed from those who did not by exhibiting significantly greater exacerbation of symptoms premenstrually and lesser morbidity postmenstrually. While water retention, negative affect, and pain were the three symptom clusters associated with the most severe ratings, the majority of women suffered from a mixture of physical, psychological, and behavioral complaints, and it was not possible to subdivide the study group by the type of symptoms. (*AM J OBSTET GYNECOL* 1986;155:277-82.)

Key words: Premenstrual syndrome, diagnosis, trend analysis

There is now considerable objective evidence that most if not all the body systems undergo changes in parallel with the ovarian cycle.¹ Subjective evidence is also available for these changes, since numerous symp-

toms have been reported as regularly fluctuating with the menstrual cycle (Moos RH, Baker K. Literature review and summary of the premenstrual tension syndrome. Unpublished research report, Stanford University, 1965), and these symptoms are experienced to a variable degree by the majority of women during their fertile years.² When severe, this collection of symptoms constitutes the premenstrual syndrome.³

Perhaps because of greater public awareness, an increasing number of women seek help for such premenstrual complaints. In the absence of specific bio-

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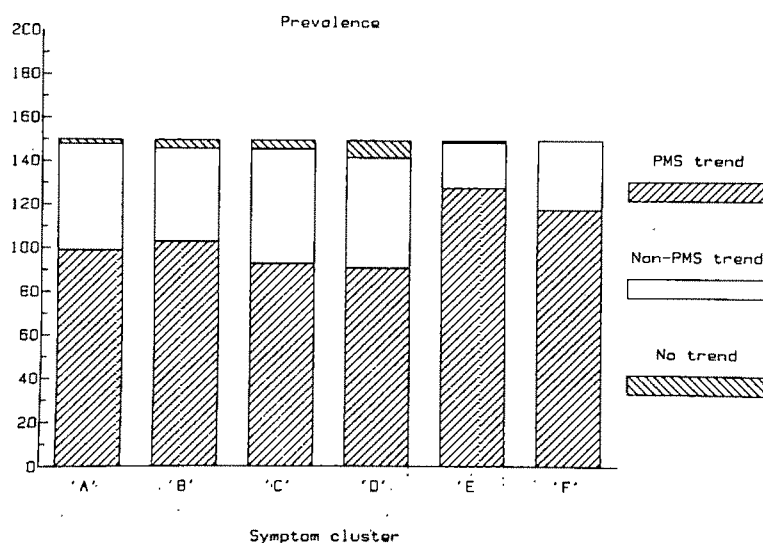


Fig. 1. Pattern of symptom trends assessed by Trigg's technique in 150 women complaining of premenstrual syndrome. A = Pain, B = concentration, C = behavioral change, D = autonomic reactions, E = water retention, and F = negative affect.

chemical markers,³ confirmation of the diagnosis is usually based on the history and exclusion of other conditions such as anxiety states, depression, and cyclical edema. However, the accuracy of retrospective symptom reporting in relation to the menstrual cycle has been questioned,¹ and it has been widely recommended that prospective assessment of these cyclical symptoms is essential to correct for confounding variables such as women's beliefs and expectations concerning menstruation and other biases.^{3, 6} Although there is considerable evidence for these observations in healthy women, who are often volunteers, there is little prospective information about the symptom patterns in women specifically complaining of premenstrual distress.

As the second part of a study into the symptom characteristics of women complaining of premenstrual syndrome, we would like to present the qualitative and quantitative results of time series analysis of the daily symptom records of women complaining of this condition with use of Trigg's technique for trends.

Patients and method

Patients. The study group consisted of the first 150 women attending the Premenstrual Tension Clinic at Dulwich Hospital who completed a standard pretreatment assessment protocol and whose symptoms for at least the preceding 6 months fulfilled our definition of premenstrual syndrome: "Distressing physical, psychological and behavioural symptoms, not caused by organic disease, which regularly recur during the same phase of each menstrual (or ovarian) cycle, and which significantly regress or disappear during the remainder of the cycle."³

The 150 women were drawn from an initial cohort of 161, of whom nine did not fulfill the above definition on first interview and two failed to complete the subsequent assessment adequately.

The average age of the group was 34.3 years (range: 22 to 49). All were menstruating regularly (mean cycle length: 29.3 days, SEM 0.27). The women were otherwise healthy, and none were taking drugs that influence ovarian activity. The majority (86.0%) had received conventional treatments for premenstrual syndrome in the past but stopped all therapy after their first visit to the clinic.

Assessment of symptoms. Before the start of therapy, all suitable patients were screened prospectively with use of a modified menstrual distress questionnaire.⁷ Our questionnaire consisted of 34 of the commonest adverse symptoms reported by Moos as fluctuating with the menstrual cycle and which are typical of premenstrual syndrome, grouped into six symptom clusters by factor analysis (pain, concentration, behavioral change, autonomic reactions, water retention, and negative affect).⁸ The symptoms were scored daily during at least one menstrual cycle on a scale of 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) to provide a serial index of how the women had been feeling in the previous 24 hours. Although the Moos questionnaire was not designed specifically for monitoring the symptoms of premenstrual syndrome and gives no clue to pathophysiology, it remains one of the most widely used psychometric tools in this field and was therefore chosen for this study.

The daily symptom records were individually analyzed by a modified Trigg's technique for trends with use of a smoothing constant of 0.25 as described pre-

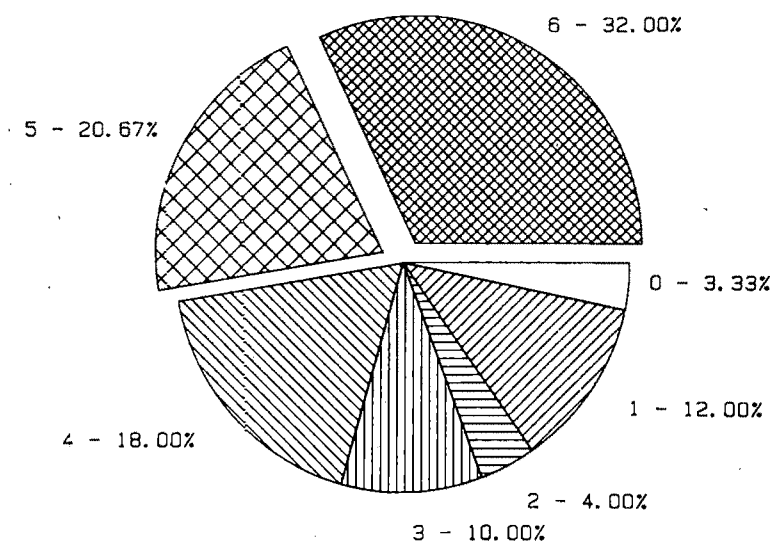


Fig. 2. Number of adverse symptom clusters showing premenstrual syndrome trends for each member of the study group.

Table I. Conditional probability of showing premenstrual syndrome trends for the six adverse symptom clusters

Symptom cluster with premenstrual syndrome trends	Probability of symptom cluster also showing premenstrual syndrome trends					
	a	b	c	d	e	f
a. Pain	—	0.80	0.76	0.75	0.91	0.86
b. Concentration	0.77	—	0.80	0.71	0.92	0.90
c. Behavioral change	0.81	0.88	—	0.74	0.89	0.88
d. Autonomic reactions	0.81	0.80	0.76	—	0.91	0.88
e. Water retention	0.70	0.74	0.65	0.65	—	0.84
f. Negative affect	0.72	0.79	0.69	0.68	0.92	—

Refer to text for definition of trend patterns.

viously.⁹ Each symptom cluster was assessed qualitatively for the pattern of symptom trends during the menstrual cycle; in order to allow for equilibration and to minimize the chance of Type II errors, trends during the first 7 days of observations were ignored. This allowed for the classification of the symptoms into one of three trend patterns:

1. *PMS trends.* Significant ($p < 0.05$) symptom trends that conformed to our definition of premenstrual syndrome⁹ with positive trends (equivalent to symptoms worsening) at some time during the 14 days preceding menstruation and negative trends (equivalent to symptoms improving) postmenstrually.

2. *Non-premenstrual syndrome trends.* The presence of significant ($p < 0.05$) trends but not conforming to our definition of premenstrual syndrome, with most commonly the presence of positive trends early in the cycle.

3. *No trends.* The absence of any significant ($p < 0.05$) positive or negative trends during the menstrual cycle.

The symptom clusters were also quantified and

symptom profiles constructed in terms of the maximum and minimum exponentially smoothed average ratings, or ESA(max) and ESA(min) respectively, the mean daily scores per menstrual cycle, and the menstrual cycle ratio (MCR), defined as ESA(max):ESA(min).⁹

Statistics. Quantitative data derived by Trigg's trend analysis was analyzed by Student's unpaired *t* tests.

Results

Qualitative assessment. The majority of the 150 patients studied by trend analyses showed symptom trends consistent with our definition of premenstrual syndrome. Depending on the symptom cluster studied, the prevalence of premenstrual syndrome trends ranged from 91 (60.7%) for autonomic reactions to 128 (85.3%) for water retention (Fig. 1). Conversely, non-premenstrual syndrome trends were detected in 21 to 53 (14.0% to 35.3%) of the records, while absent trends were the least common, occurring in only 0 to 8 (0% to 5.3%) of the women (Fig. 1). Overall, all the women were found to have at least one symptom cluster with

Table II. Quantitative assessment of the symptoms of women with and without premenstrual syndrome

Symptom cluster	Scores during menstrual cycle						Statistics for a vs b		
	MCR				Mean	SD	MCR		Mean
	ESA (max)		ESA (min)				ESA (max)	ESA (min)	
	Mean	SD	Mean	SD					
Pain									
a	1.25	(0.31)	0.42	(0.16)	0.69	(0.35)	+	—	+
b	0.87	(0.23)	0.51	(0.10)	0.67	(0.37)	<0.001	<0.01	NS
Concentration									
a	1.05	(0.23)	0.20	(0.15)	0.49	(0.34)	+	—	+
b	0.49	(0.08)	0.27	(0.07)	0.32	(0.36)	<0.001	<0.01	<0.05
Behavioral change									
a	0.92	(0.24)	0.18	(0.11)	0.43	(0.29)	+	—	+
b	0.48	(0.07)	0.31	(0.07)	0.38	(0.48)	<0.001	<0.001	NS
Autonomic reactions									
a	0.55	(0.14)	0.07	(0.06)	0.26	(0.28)	+	—	+
b	0.31	(0.07)	0.14	(0.03)	0.24	(0.28)	<0.001	<0.001	NS
Water retention									
a	1.32	(0.29)	0.27	(0.24)	0.66	(0.38)	+	—	+
b	0.78	(0.15)	0.34	(0.13)	0.41	(0.35)	<0.001	<0.02	<0.05
Negative affect									
a	1.29	(0.36)	0.33	(0.17)	0.67	(0.39)	+	—	—
b	1.16	(0.15)	0.56	(0.18)	0.84	(0.50)	<0.05	<0.001	NS

Symptoms scored on a scale of 0 to 3 (no symptoms to severe symptoms). MCR = Menstrual cycle ratio—ESA (max):ESA (min). ESA (max) = Maximum exponentially smoothed average. ESA (min) = Minimum exponentially smoothed average. a = Women with premenstrual syndrome trend. b = Women without premenstrual syndrome trends. + = a > b. — = b > a.

significant trends at some time in the cycle. Over 80% of the women were found to have premenstrual syndrome trends for three or more of the symptom clusters, with almost one third (32.0%) showing this pattern for all six, while only five (3.3%) failed to have premenstrual syndrome trends for any of the clusters studied (Fig. 2).

If a particular symptom cluster demonstrated premenstrual syndrome trends, there was a 65% to 92% probability of one of the other clusters also showing similar trends (Table I). The strongest associations were with water retention (89% to 92%) and negative affect (84% to 90%), and the weakest with autonomic reactions (65% to 75%). Physical and psychological subtypes of premenstrual syndrome could not be distinguished, since only 15 (10.4%) of the 145 women with premenstrual syndrome trends for at least one symptom cluster showed these trends for purely the physical clusters (pain, autonomic reactions, and water retention) and only five (3.4%) for purely the psychological and behavioral ones (concentration, behavioral change, and negative affect); the remaining 125 (86.2%) women presented with a mixture of symptoms. Similarly there was no significant difference in the timing of the waxing and waning of the various symptoms; on average, significant positive trends started 6 to 8 days before menstruation, whereas significant negative trends began at 7 to 9 days following the onset of bleeding.

Quantitative assessment. Irrespective of the pattern

of trends, water retention, negative affect, and pain were responsible for the most morbidity and autonomic reactions the least as judged by ESA(max) and mean daily scores per menstrual cycle (Table II); although both these variables appear to show relatively low values for all symptom clusters, this is inherent in the periodic nature of the data and the effect of "smoothing." For each symptom cluster, ESA(max) was significantly greater ($0.05 < p < 0.001$) and ESA(min) significantly smaller ($0.02 < p < 0.001$) for the women with premenstrual syndrome trends compared with the rest. As a typical example, this difference is depicted graphically for the symptom cluster pain in terms of the raw daily symptom ratings (Fig. 3). The graph shows that on average the 99 women with premenstrual syndrome trends tended to have lower postmenstrual and higher premenstrual ratings, and thus greater swings in symptoms during the menstrual cycle, than the 51 who recorded either non-premenstrual syndrome trends or no trends. The mean daily symptom scores tended to be higher for the women with premenstrual syndrome trends except for negative affect, which was the symptom cluster associated with the greatest degree of complaints by the women with non-premenstrual syndrome or no trends (Table II).

Comment

The present investigation shows that at least in our selected group of clinic attenders, a complaint of pre-

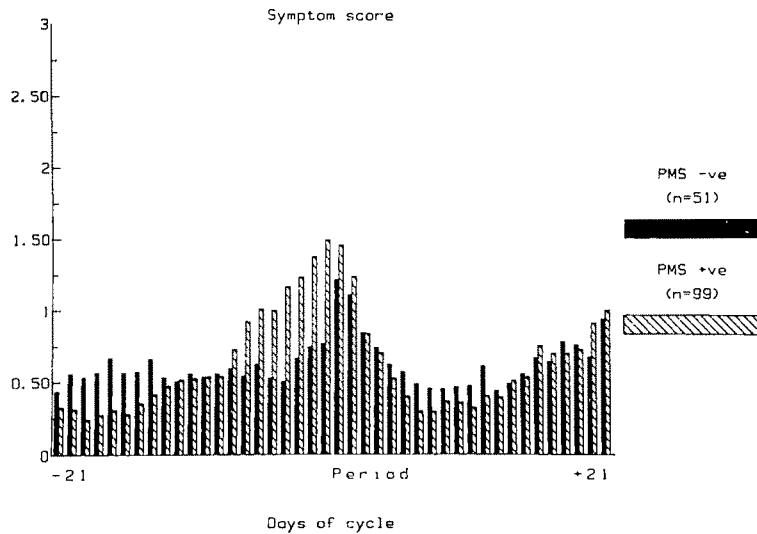


Fig. 3. Average daily scores for the symptom cluster "pain" for the groups with and without premenstrual syndrome trends. Symptoms scored on a scale of 0 to 3 (no symptoms to severe symptoms).

menstrual syndrome that satisfies our preliminary definition is usually sufficient proof of significant exacerbations of at least some symptoms before menstruation. Thus on daily prospective symptom rating there was a greater than 95% probability of finding at least one and an over 80% probability of finding three or more of the six symptom clusters with significant trends during the menstrual cycle consistent with the syndrome. It therefore seems that in this population of women, made aware of the menstrual cycle by adverse complaints, retrospective reporting is a more accurate index of cyclical symptoms than in the case of healthy, and perhaps less motivated, subjects.^{4, 10}

Quantitative assessment of the symptom records of women with and without premenstrual syndrome trends for a particular symptom cluster shows that the overall severity of symptoms, as judged by the mean daily scores, is largely comparable. Instead, trend analysis differentiates women who complain of the syndrome in terms of the degree of change in symptoms during the menstrual cycle. Women found to have premenstrual syndrome trends tend to complain of relatively more severe symptoms premenstrually with considerable improvement with menstruation. In contrast, the symptoms of women without premenstrual syndrome trends tend to fluctuate to a lesser degree and particularly do not regress to the same extent postmenstrually (Fig. 3). This suggests that the primary difference between these two groups of women with apparently similar complaints may lie in the greater chronic morbidity manifested by those whose symptom profiles do not fulfill the diagnostic criteria for premenstrual syndrome, rather than in the severity of premenstrual symptoms. This disparity is expressed numerically by the MCR, which defines both the degree

of fluctuation in symptoms during the menstrual cycle, $ESA(max)-ESA(min)$, and also baseline symptomatology, $ESA(min)$.

The symptoms that were both the most prevalent and distressing for the women found to have premenstrual syndrome trends involved a physical and a psychological cluster, namely, water retention (weight gain, rashes, breast tenderness, swelling) and negative affect (crying spells, loneliness, anxiety, restlessness, irritability, mood swings, depression, tension). This, and the finding that the majority of women with premenstrual syndrome trends showed premenstrual exacerbations for physical, psychological, and behavioral parameters on a similar time scale, suggests that, irrespective of symptoms, the primary etiologic mechanism for the syndrome is similar in all cases. This adds support to the concept that the common denominator in the premenstrual syndrome is ovarian activity and ovulation,^{11, 12} which, as noted earlier, is known to be associated with changes affecting most body systems.¹ Women complaining of the syndrome probably have heightened sensitivity to these changes with resultant exaggerated symptoms.

In conclusion, this study demonstrates the practical applications of trend analysis for the study of menstrually related phenomena, including the diagnosis and assessment of premenstrual syndrome. It shows that, in this group of women, retrospective history that fulfills our diagnostic criteria is usually adequate proof of significant premenstrual distress for at least some symptoms. Trend analysis can, however, be applied to uncertain cases and be used to identify specific cyclical symptoms, so it represents an objective method of both qualitative and quantitative assessment. The confirmation by this sensitive technique of the multisymptom

nature of premenstrual syndrome in the majority of complainers makes it unlikely, in our view, that one particular biochemical or psychological abnormality can explain all symptoms in any individual.

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Reimplantation of a human embryo with subsequent ovarian pregnancy

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An ovarian pregnancy followed ovulation induction and in vitro fertilization. The patient had multiple risk factors, including two previous tubal pregnancies, endometriosis, and use of an intrauterine contraceptive device. (*AM J OBSTET GYNECOL* 1986;155:282-3.)

Key words: Ovarian pregnancy, ovulation induction, in vitro fertilization

A 30-year-old woman, gravida 2, para 0, with two ectopic pregnancies, presented with clinical signs and symptoms of a third ectopic pregnancy.

Case report

On November 20, 1984, human menopausal gonadotropin (hMG) (Pergonal, Serono Laboratories), 75 IU of follicle-stimulating hormone and 75 IU of luteinizing hormone per ampule, was administered to this patient. Induction of ovulation was initiated at three ampules per day. Twenty-four ampules were given during an 8-day period. On the tenth day, at an estradiol level of 1350 pg/ml, she was given 10,000 IU of human chorionic gonadotropin (hCG) (Profasi, Serono).

At the time of laparoscopy, 34 hours later, four oocytes were recovered from the right ovary. The right tube and the right ovary were well visualized. The left tube was partially visualized. There were adhesions in-

volving both tubes and ovaries. Tubal fimbria were only visualized on the right. Only 10% of the left ovary could be seen. The cells were fertilized and incubated according to the technique reported by Garcia et al.¹

She was given 12.5 mg of progesterone intramuscularly daily starting the second day after laparoscopy. Three days after laparoscopy, two of the fertilized ova that had achieved a four-cell size were inserted transcervically again by the method of Garcia et al.¹ Ten days after the in vitro fertilization, she reported an episode of vaginal bleeding.

Twenty days later she presented to the emergency room complaining of sharp left lower quadrant pain and dizziness. Ten milliliters of nonclotting blood was obtained by culdocentesis. The pregnancy test was positive.

At laparotomy, a 3 by 5 cm hemorrhagic left ovarian pregnancy was found and excised by wedge resection without complications. The patient was discharged in good condition on the fourth postoperative day. The pathology report confirmed the surgical findings of an ovarian pregnancy. Pregnancy test at the 4-week postoperative visit was negative.

This patient had many risk factors for ovarian preg-

nancy, including a left tubal pregnancy that was treated with a linear salpingectomy, bilateral ovarian wedge resections, endometriosis, laparotomy for lysis of adhesions, and use of an intrauterine contraceptive device, (copper 7, G. D. Searle & Co.) for 3 years.

The original report of Steptoe and Edwards of in vitro fertilization described a case that resulted in a tubal pregnancy. This is the first reported case of ovarian pregnancy after in vitro fertilization. The exact mechanism for the ovarian pregnancy is unclear. The patient did have coitus with use of a condom 2 days before the laparoscopy. At least one of the tubes of the patient was probably patent. The reverse migration of an embryo from the uterus out through the fallopian

tube to the ovary seems unlikely. However, the reverse migration of a fertilized ovum was the mechanism postulated by Steptoe and Edwards² for the tubal pregnancy that followed in vitro fertilization.

Further data must be accumulated to determine if the risk of ovarian pregnancy is increased after ovulation induction and in vitro fertilization.

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Pulsatile heparin administration in pregnancy: A new approach

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The role of anticoagulation during pregnancy for women with a past history of thromboembolism is a controversial issue. The risk of recurrence of thromboembolism, however, is of the magnitude of about 15%, which warrants intervention, especially when one is dealing with such a potentially lethal disease. The analysis of plasma heparin levels is suggested as the best way to monitor treatment. As an alternative to subcutaneous injections twice or three times daily, 15 women used a portable infusion pump (Zyklomat, Ferring) that delivered heparin either subcutaneously or intravenously for periods of up to 25 weeks. There were no recurrences of thromboembolism and no clinical signs of osteoporosis. The patient acceptability of the pump was excellent as was the ability to maintain adequate heparin levels. Although there are some theoretical benefits with intermittent infusion of heparin evaluation of the advantages from a medical point of view must await further studies. (*AM J OBSTET GYNECOL* 1986;155:283-7.)

Key words: Heparin, pregnancy, pulsatile administration

In developed countries pulmonary embolism is now the most frequent cause of maternal death. Pregnancy has been called a "hypercoagulable state" and the incidence of thromboembolic episodes is clearly increased over that in the nonpregnant state.¹ There are certain risk groups in which prophylactic anticoagulation has been recommended, such as women with a history of thromboembolism occurring during a previous pregnancy or while using oral contraceptives as well as women without any apparent cause. The risk of developing a new thrombus is significant, about 12% to 13%.² Women with a heart valve prosthesis also demand continuous anticoagulation during pregnancy.³ Women

with repeated late pregnancy losses have previously been given anticoagulants perhaps on not too solid grounds. However, women with such a medical history may have circulating anticoagulants against phospholipids, and these patients may benefit from anticoagulation during pregnancy in addition to treatment with steroids.¹

Oral anticoagulants cross the placenta and give rise to malformations if given in the early gestational period.³ There is a risk of bleeding episodes in the fetus during the entire pregnancy. In Sweden the use of coumarin preparations during pregnancy is not recommended and a woman receiving such drugs who becomes pregnant is recommended to have a termination of pregnancy. Heparin does not cross the placenta and is safe for the baby. There are, however, reports of bone demineralization that seem to be related to the dose and duration of heparin administration.⁶ The mechanism of heparin-induced osteoporosis

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Table I. Clinical data

Subject	Age (yr)	Gravidity and parity	Thrombosis			Start of treatment (pregnancy week)
			During current pregnancy	During previous pregnancy or with oral contraceptives	Outside pregnancy	
E. T.	33	G1	×			24
E. P.	32	G2, P1	×			22
L. W.	22	G1	×			12
G. K.	36	P1	×			p.p.
E. J.	29	P1	×			p.p.
E. H.	25	G2, P0	×			14
N. N.	24	G1	×			20
M. W.	22	G2, P0		×		11
B. I.	36	G2, P1		×		9
B. M.	34	G3, P1		×		8
E. T.	33	G2, P1		×		6
S. B.*	31	G1		×		15
S. A.	28	G4, P3		×		14
C. J.	23	G1		×		11
E. J.	31	G2, P1		×		14
S. K.	42	G1			×	19
P. O.	33	G3, P2			×	15
L. S.†	34	G1			×	7

SVD, Spontaneous vaginal delivery. CS, cesarean section.

*Woman with an antithrombin deficiency (30% to 40% of normal values). Baby normal.

†Woman with a heart valve prosthesis. Supplementary medication with acetylsalicylic acid and, after week 18, dipyridamole, 75 mg × 4.

is not known, and neither is the incidence of clinical symptoms due to osteoporosis. Hellgren (personal communication) reported on two patients with compression fractures in 100 treated patients. In a controlled trial one of 20 patients developed severe osteopenia.⁷ On the other hand, one woman in the control group developed a deep vein thrombosis.

There is a definite need to improve anticoagulation therapy during pregnancy. This might be achieved through recent advances in heparin structure research.⁸ It is conceivable that small heparin fragments with different properties compared with those of the complete heparin molecule may be used during pregnancy, but the safety and efficacy during pregnancy have not as yet been verified. Another way of improving anticoagulation is to monitor the heparin dose more individually instead of giving a fixed dose. A commonly used dose is 10,000 IU subcutaneously twice daily.⁷ This dose may be too large in some patients and too small for some patients, as the individual response varies greatly. The use of clotting times, such as the activated partial thromboplastin time, either just before the injection or between the injections has been advocated but with prophylactic doses of heparin this method is very insensitive. During the past 2 years patients requiring anticoagulation therapy have been monitored with plasma heparin levels. The women have also been offered participation in a trial of pulsatile heparin administration with a portable infusion pump (Zyklomat, Ferring). Although there are previous case reports of

continuous heparin administration during pregnancy,⁹ this is the first report in which a number of women, using an infusion pump, had plasma heparin levels monitored throughout pregnancy.

Material and methods

Since March, 1983, 18 women have been treated therapeutically and/or prophylactically with heparin during pregnancy. Pertinent data on the women, the reasons for heparin administration, the heparin dosage, and the duration of therapy are listed in Table I. Fifteen women used the portable infusion pump for periods varying from a few days to 25 weeks. Six women used the subcutaneous route only, and five used the intravenous route only, whereas four women used both administration techniques. Three women decided to discontinue the pulsatile administration after only a short time. In one case the reason was thrombophlebitis; in another case it was an incorrectly placed catheter (on the dorsal side of the hand instead of the volar side of the arm), and in another case it was because the patient considered the pump inconvenient. Serial determinations of plasma heparin concentrations after a subcutaneous injection of 10,000 IU of heparin were performed in all women to determine the adequate dose. In women using the portable pump heparin determinations were made at each refill, usually at 10-day intervals. In women using the subcutaneous route heparin determinations were performed at regular intervals that were more frequent as the pregnancy pro-

Heparin dosage ($\times 10^3$ IU)			Duration of continuous/ total therapy (wk)	Mode of delivery
Subcutaneous	Continuous			
	Subcutaneous	Intravenous		
—	20	20	16/16	SVD
20	20-25	—	6/7	CS
20	20	15-17,5-20	12/26	SVD
20	20	20	4/7	—
20	20	—	3/5	—
20	—	20	23/25	CS
20	—	—	—/14	SVD
20-25	20	—	1/29	SVD
10-15	—	10	1/31	CS
20	20	—	6/32	SVD
20	—	15-20	25/34	SVD
20-25	20	—	3/25	SVD
20-30	—	20	1/27	SVD
20	—	—	—/31	CS
20-25	20	—	13/27	SVD
20-30	30	30	8/16	CS
10-15	—	—	—/24	SVD
15-20	20	—	4/32	SVD

ceeded. In these cases samples were taken immediately before and 2 to 4 hours after the subcutaneous injection. In all women regular checks of antithrombin concentrations were made. Platelet counts were performed in late pregnancy and before delivery. In patients receiving subcutaneous injections the heparin dose was reduced at the time of delivery to 5,000 IU twice daily. For patients using a pump no changes were made. In the postpartum period the women continued heparin administration at a lower dose, for instance, 5,000 to 7,500 IU twice daily together with Waran tablets until prophylactic levels, 10% to 25% in Simplastin-A, were reached. Oral anticoagulation was continued 6 to 8 weeks for the patients receiving prophylaxis and for individually determined periods for those who developed thrombosis during the actual pregnancy. The mothers were allowed to breast-feed.¹⁰

The Zyklomat pump works on the principle of fluid transport in a tube with progressive squeezing in one direction. Initially only the model with a fixed interval of 90 minutes was available. This model conveys 50 μ l of solution per stroke during the 1-minute running time and thus delivers $16 \times 50 \mu\text{l} \times 25,000$ IU/ml or 20,000 IU of heparin daily. Later in the study period a new pump with programmable pause intervals was made available. This pump makes it possible to adjust the daily dose according to the needs of the patient. Dilution of the heparin solution with sterile water did not give reproducible results when tested in vitro (unpublished results) and this method of varying the heparin dose was therefore abandoned. Blood for heparin determination was taken by venipuncture, collected on ice, and centrifuged at 13,000 rpm for 15 minutes in a cooler centrifuge. The samples were frozen until an-

alyzed, with analysis done once a week. Heparin levels of 0.10 to 0.20 IU/ml were considered to be ample for prophylaxis, compared with therapeutic levels, which are between 0.20 and 0.70 IU/ml. An anti-Xa method with the use of the chromogenic substrate S 2222 was used.¹¹

Results

There were no recurrences of thrombosis during heparin prophylaxis. Routine roentgenography was not used in this series to detect any subclinical osteoporosis, but no overt symptoms attributable to osteoporosis developed in any patient. Of the 16 babies delivered, 11 were born after a normal vaginal delivery and five after cesarean section performed for the usual obstetric reasons.

There were no bleeding episodes during vaginal delivery or cesarean section that were attributable to the use of heparin. One woman developed a vaginal hematoma due to an arterial bleeding. No complications attributable to anticoagulation appeared in the children except for one woman (S. K.) who had used oral anticoagulation during her first 19 weeks of pregnancy. In this baby certain stigmata that are associated with early oral anticoagulant therapy were found.

The heparin levels after subcutaneous injections of 10,000 IU are shown in Fig. 1. The large interindividual responses are noteworthy. In some women adequate concentrations are maintained for almost 12 hours, whereas in other women therapeutic levels are reached with inherent bleeding risks. In some women undetectable heparin levels are reached after just 6 to 8 hours and these women actually require three daily injections for "around the clock" anticoagulation.

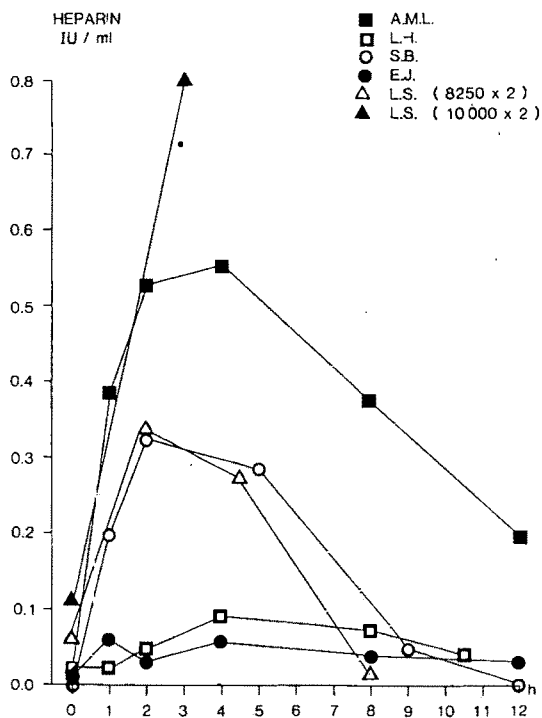


Fig. 1. Heparin levels after subcutaneous injections of 10,000 IU of heparin.

Some results of sampling from patients using the pump are shown in Fig. 2. The heparin levels are well within intended levels. An example of multiple testing during one pulse interval is shown in Fig. 3. When the pump with programmable intervals was used, in some cases the daily dose could be reduced with maintained anticoagulation (Fig. 4).

Initially the subcutaneous route of administration was used with the pump. The incidence of hematomas that necessitated a change of the insertion site was not negligible (two of 10 cases) and prompted a change to the intravenous route. Although the refill procedure was more complicated, this route proved to be superior. The acceptability to the women was excellent; the pump was in fact preferred to daily subcutaneous injections by most of the women. Common remarks were: "It makes me free and independent of needles and syringes." "It doesn't hurt." The inconvenience, for instance, when taking a shower, was easily solved with plastic bags used for protection.

Comment

When a woman has had a deep venous thrombosis during a previous pregnancy or while using oral contraceptives, the risk of recurrence motivates prophylaxis with anticoagulants. Antiplatelet drugs are not very effective against venous thrombosis¹² and as oral coumarin derivatives cross the placenta they should

never be used during pregnancy. The use of physical methods such as supportive stockings has not been evaluated for its efficacy. Heparin thus remains the drug of choice for prophylaxis. It is important to individualize the dose through careful monitoring, which at least initially should include serial measurements of plasma heparin levels to find the lowest dose that gives 24-hour anticoagulation. Depending on individual response this sometimes would require three daily subcutaneous injections. Such women in particular are candidates for heparin administration with a portable pump, which gives levels that fluctuate less and with which a reduction of the total dose is often possible. It is also conceivable that unnecessarily high peak heparin levels sometimes seen after subcutaneous injections could influence the rate of demineralization, but this has to be confirmed in further studies. The patient compliance has been excellent and most women would choose the pump compared with subcutaneous injections in a subsequent pregnancy.

Demineralization, the disadvantage with heparin, has previously restricted heparin prophylaxis to the postpartum period.¹³ Of the 26 women in that study, however, one developed a pulmonary embolus in the twenty-third week of pregnancy. Women who develop thromboembolic episodes either without any apparent cause or after an operation and in whom a coagulation analysis is quite normal probably do not need any prophylaxis during a subsequent pregnancy. However, it seems unjustifiable to deny the only effective prophylaxis to women who had a thrombosis during a previous pregnancy or while using oral contraception. The slight risk of clinically overt demineralization must be weighed against the very definite risk of a potentially deadly disease. As the demineralization is probably dose-dependent, an individually adjusted dose, varied as pregnancy proceeds, is a prerequisite to keep the dose as low as possible. In this context administration of pulsatile minidoses of heparin is an interesting alternative to the usual subcutaneous injections. The advantages from a medical point of view must await further studies that are presently under way.

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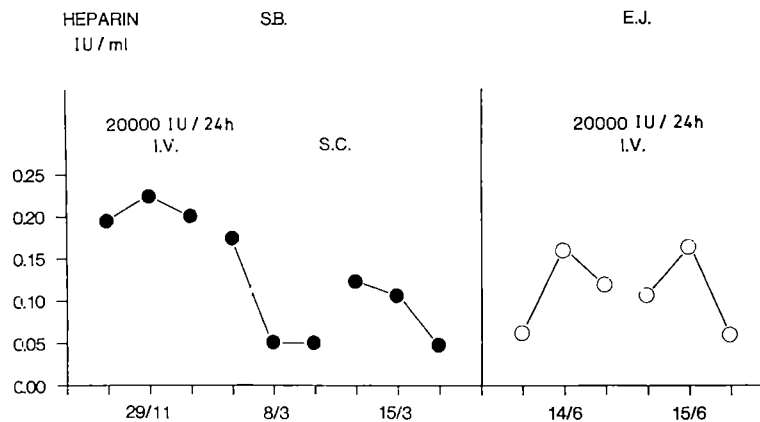


Fig. 2. Heparin levels in women using the pump for subcutaneous and intravenous administration (S. B.) and intravenous administration (E. J.).

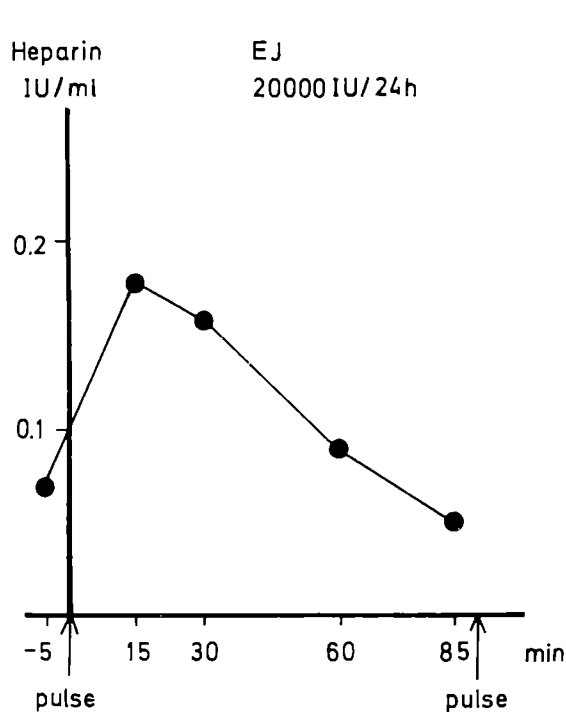


Fig. 3. Heparin levels during one pump interval (intravenous administration).

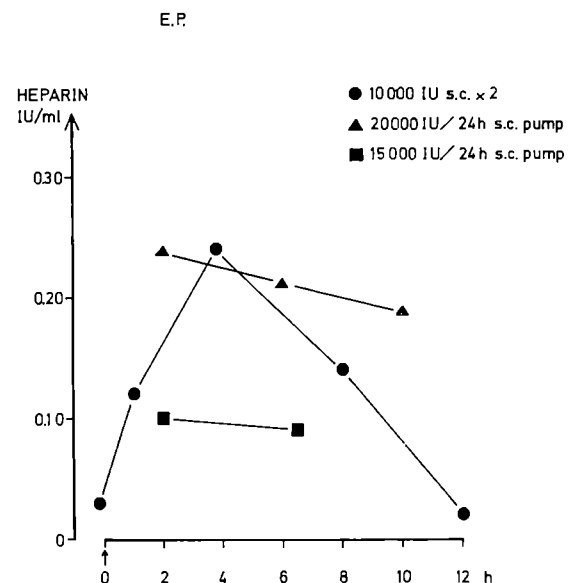


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Urinary diversion with use of ileal and sigmoid conduits

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Two hundred eighteen patients underwent urinary diversion: 156 with ileal and 62 with sigmoid conduits. There were no significant differences between the two groups regarding frequency of conduit morbidity or patient survival. The ileal conduit is preferred for urinary diversion with anterior exenteration, whereas the sigmoid conduit is preferable for urinary diversion with total exenteration. (AM J OBSTET GYNECOL 1986;155:288-92.)

Key words: Urinary diversion, ileal conduit, sigmoid conduit, anterior exenteration, total exenteration

The most frequent operation for urinary diversion has involved the ileal conduit. The use of an isolated segment of ileum was first reported by Seiffert,¹ in 1935, and was popularized later by Bricker,² in 1950. Bricker demonstrated the advantage of using an isolated segment of ileum as a urinary conduit. A variation of the Bricker operation, with the use of an isolated segment of sigmoid colon, was first reported by Mogg,³ in 1965, as a treatment of neurogenic urinary incontinence and later by Symmonds and Gibbs,⁴ in 1970, as a means of urinary diversion after radical gynecologic surgery. Using a segment of sigmoid colon in total exenteration obviates the need for an intestinal anastomosis, making sigmoid urinary diversion especially advantageous for the irradiated patient undergoing total exenteration. In either case, the intestinal segment is kept as short as possible to provide rapid runoff and to minimize the contact between urine and the intestinal mucosa so the conduit does not become a reservoir.

Our series

Between 1955 and 1981, 218 urinary conduit procedures were performed by members of the Division of Gynecologic Surgery at the Mayo Clinic. The ileum was used in 156 (72%) and the sigmoid colon in 62 (28%). No patient underwent a primary urinary diversion with an isolated segment of transverse colon. Patients having previous uretersigmoidostomies into an intact colon and undergoing revision to an ileal or a sigmoid conduit were excluded from the series. The purpose of this study was to determine if there are

major physiologic or functional differences between ileal and sigmoid conduits or whether similar results can be obtained regardless of the segment of intestine used.

Methods

Patients were categorized according to whether they had an ileal or a sigmoid colon conduit. Preoperative factors such as the original disease and treatment, the indications for urinary diversion, the urologic status, and the patient's age and weight were tabulated.

All patients underwent preoperative excretory urography. Excretory urography was also done before dismissal from the hospital, within 6 months after dismissal, and subsequently at 12-month intervals or as necessary.

Postoperative factors considered included metabolic change in excretory function manifested by hyperchloremic acidosis or transient acidosis, chronic pyelonephritis, and calculi formation. Problems directly related to the urinary conduit, such as urinary leakage (ureteral or conduit), ureteral obstruction, stomal necrosis, and retraction with stenosis, were assessed. In addition, other postoperative complications such as pelvic abscess, wound infection, ileus, phlebitis, and pulmonary embolism were considered.

The postoperative period was defined as 60 days from the time of operation. Follow-up of patients was usually by return visit to the clinic if the patient was alive or by letter to the patient or the physician if the patient had not recently visited the clinic.

The urinary conduits were constructed by use of a modification of the Bricker method.⁵ The technique to construct a sigmoid conduit is similar to that used to construct an ileal conduit, although less complex because an ileoileostomy is obviated. After isolation of a segment of either the ileum or the sigmoid colon, the proximal end of the intestine is closed with a through-

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Table I. Original conditions leading to urinary diversion

Condition	Ileal conduit	Sigmoid conduit	Total
Cervical carcinoma	103	42	145 (67%)
Vaginal carcinoma	11	14	25
Urethral carcinoma	6	0	6
Vaginal melanoma	3	1	4
Vaginal sarcoma	3*	2*	5
Bladder carcinoma	4	0	4
Endometrial carcinoma	12	1	13
Vulvar carcinoma	5	0	5
Colon carcinoma	4	0	4
Other	5†	2‡	7
Total	156	62	218

*Includes one patient with botryoid sarcoma.

†Includes patients with exstrophy of bladder, meningo-myelocele, radiation-related vesicovaginal fistula, choriocarcinoma, and recurrent squamous carcinoma from malignant degeneration of ovarian dermoid.

‡Both patients had recurrent carcinoma of ovary.

and-through continuous catgut suture supplemented by a second row of interrupted fine silk sutures. This portion of the conduit rests naturally in position above the promontory, where ureters, having been divided at or above the pelvic brim and common iliac arteries, are anastomosed to the intestine with a single row of interrupted 3-0 or 4-0 chromic catgut suture; thus the full thickness of ureter is fixed to the full thickness of intestine. Stenting of the ureterointestinal anastomosis was not necessary in our series, and no effort was made to produce a nipple or valve to prevent reflux. The peritoneal flap associated with the ureter was attached to the serosa of the conduit beyond the ureterointestinal anastomosis to further secure a tension-free union. The serosa of the proximal end of the conduit was fixed to the fascia immediately above the pelvic brim. Then a 3 to 4 cm ring of tissue was excised from all layers of the abdominal wall so the distal end of the conduit could be brought out a preselected stomal site, usually in the right lower quadrant of the abdomen. The skin apertures for a sigmoid conduit were somewhat larger than those made for an ileal conduit.

Results

The original condition leading to urinary diversion was cervical carcinoma in 145 patients (67%) (Table I). Malignancy of the vagina, bladder, endometrium, vulva, and colon led to the need for urinary diversion in 66 patients (30%), and seven patients (3%) underwent urinary diversion for other conditions.

Treatment preceding urinary diversion varied considerably. Most patients with recurrent carcinoma did not have initial radiation therapy or operation at the Mayo Clinic. Sixty-seven percent of patients had re-

Table II. Treatment preceding urinary diversion

Treatment	Ileal conduit	Sigmoid conduit	Total	
			No.	%
Radiation therapy	97	50	147	67
Operation	22	1	23	11
None*	37	11	48	22
Total	156	62	218	100

*Urinary diversion was part of primary treatment.

Table III. Intestinal segments used in urinary diversion

Procedure	Ileum	Sigmoid	Total	
			No.	%
Anterior exenteration	111	4	115	53
Total exenteration	38	58	96	44
Urinary diversion alone	7	0	7	3
Total	156	62	218	100

ceived prior radiation therapy, 11% had prior operation, and 22% had urinary diversion as part of primary treatment (Table II).

Two hundred eleven patients had an ileal or a sigmoid conduit placed during anterior or total exenteration, and seven had urinary diversion as the only procedure (Table III).

The median age was 54 years (range 2 to 81) for patients receiving an ileal conduit and 51 years (range 1 to 74) for patients receiving a sigmoid conduit. The median weights of patients with ileal and sigmoid conduits were 61.7 and 64.5 kg (ranges 11.4 to 116.7 and 9.1 to 111.7), respectively.

The preoperative urinary status, as determined by excretory urography, was abnormal in 38 patients (24.3%) who had an ileal conduit and in 10 patients (16.1%) who had a sigmoid conduit. Postoperative excretory urograms showed similar changes of improvement, stability, or increasing abnormality between the two groups (Table IV).

Hyperchloremic acidosis requiring sodium bicarbonate therapy was seen in only three patients: one in the ileal conduit group and two in the sigmoid conduit group. Transient increases in serum chloride level >106 mEq/L were noted in 15 patients (9.6%) in the ileal conduit group and nine (14.5%) in the sigmoid conduit group; none of these 24 patients required treatment for the condition.

Eight of the 218 patients (3.6%) suffered urinary leaks or fistulas during the postoperative period (Table V). Seven of the eight patients had undergone exenterative operations for recurrent cervical carcinoma af-

Table IV. Urologic status of patients with ileal and sigmoid conduits for urinary diversion*

Group	Excretory urogram				
	Preoperative	No.	Postoperative	No.	%
Ileal conduit (156 patients)	Normal	118	Unchanged	109	92
			Abnormal	9	8
	Abnormal	38	Improved	21	55
			Unchanged	12	32
			More abnormal	5	13
Sigmoid conduit (62 patients)	Normal	52	Unchanged	48	92
			Abnormal	4	8
	Abnormal	10	Improved	5	50
			Unchanged	3	30
			More abnormal	2	20

*As determined by preoperative and postoperative excretory urography.

Table V. Conduit morbidity and postoperative complications in patients with ileal and sigmoid conduits for urinary diversion*

	Ileal conduit (156 patients)		Sigmoid conduit (62 patients)	
	No.	%	No.	%
<i>Conduit morbidity</i>				
Fistula (ureteral or conduit)	5	3.2	3	4.8
Ureteral obstruction	13	8.3	7	11.2
Stomal necrosis, stenosis, or retraction	17	10.9	10	16.1
Chronic pyelonephritis	15	9.6	11	17.7
Calculi	9	5.7	2	3.2
Total	59	37.8	33	53.2
<i>Postoperative complications</i>				
Wound infection	8	5.1	3	4.8
Pelvic abscess	6	3.8	5	8.1
Bowel obstruction	4	2.5	5	8.1
Ileus (>10 days)	7	4.5	2	3.2
Phlebitis or pulmonary embolus	3	1.9	1	1.6
Total	28	18.0	16	25.8

*No significant differences in any of the characteristics were found between the two groups.

ter irradiation, and one was treated for recurrent cervical cancer after a radical hysterectomy. Two of the eight patients died during the postoperative period, which emphasizes the seriousness of this complication.

No statistically significant differences were observed between the two groups regarding the frequency of conduit morbidity (Table V). Twenty patients (9.1%) had significant unilateral or bilateral ureteral obstruction: 13 patients with ileal conduits and seven with sigmoid conduits. In the ileal conduit group, five patients had obstruction during the postoperative period (not requiring reoperation), seven had obstruction later (associated with recurrent carcinoma), and one had obstruction related to radiation fibrosis. In the sigmoid conduit group, six had obstruction during the postoperative period and only one had obstruction later (associated with recurrent carcinoma). The obstruction

was transient in two patients, one of whom seemed to have a mucous plug and the other had ureterointestinal edema (patient would have benefited from placement of a ureteral stent at operation).⁷

Stomal necrosis, stenosis, or retraction was noted in 17 patients (10.9%) in the ileal conduit group and 10 patients (16.1%) in the sigmoid conduit group.

Chronic pyelonephritis was seen in 15 patients (9.6%) in the ileal conduit group and 11 (17.7%) in the sigmoid conduit group. Approximately 50% of these patients had urologic evidence of pyelonephritis before urinary diversion, and the condition stabilized with aggressive antibiotic therapy that did not require conduit revision or nephrectomy in any patient. Actual calculi were found in the upper urinary tract in six patients (3.8%) in the ileal conduit group and in one patient (1.6%) in the sigmoid conduit group. Calculi occurred within the

Table VI. Status of patients with ileal or sigmoid conduits for urinary diversion

Status	Ileal conduit (156 patients)		Sigmoid conduit (62 patients)	
	No.	%	No.	%
Alive	66	42.3	25	40.3
Dead				
Persistent cancer	33	23.1	17	27.4
Recurrent cancer	23	17.9	10	16.1
Renal failure	4	2.6	1	1.6
Unrelated cause	13	8.3	4	6.5
Postoperative*	6	3.9	3	4.9
Unknown	3	1.9	2	3.2
Total	156	100.0	62	100.0

*Within 60 days after operation.

conduit itself in three patients (1.9%) with ileal conduits and in one (1.6%) with a sigmoid conduit.

The incidences of pelvic abscess, wound infection, phlebitis, pulmonary embolus, and ileus were as expected and did not differ significantly between the two groups (Table V).

Nine patients died during the postoperative period (60 days after operation). All had an underlying malignancy and prior pelvic irradiation. Six patients died of intestinal obstruction with bowel necrosis and sepsis (one also had a conduit leak), two of pulmonary embolus, and one of pneumonia.

Fistula (ureteral or conduit) occurred in eight patients; seven had prior pelvic irradiation. Unilateral or bilateral ureteral obstruction occurred in 20 patients; in 11 it was related to radiation fibrosis, in seven it was related to recurrent cancer, and in two operation was the only predisposing factor. Likewise, stomal necrosis, stenosis, or retraction was a significant problem in 27 patients, and 24 had prior pelvic irradiation.

Survival from 30 days after operation to death from all causes was similar in both groups (Fig. 1). The status of all patients at the time of their last follow-up visit or death is shown in Table VI.

Comment

The successful outcome of exenterative operations is highly dependent on successful urinary diversion.⁶ The preoperative factors that appear to predispose patients to increased postoperative morbidity related to urinary diversion are underlying malignancy and prior pelvic irradiation.⁷

Conduits of ileum or of sigmoid and transverse colon have been used without significant physiologic differences among these bowel segments.⁸⁻¹⁰ The jejunum has been used, but it is associated with a greater risk of electrolyte imbalance.^{11,12} The risk of electrolyte disturbance increases with impairment of renal function.

Bricker¹³ reported that neither ileal nor colon segments present electrolyte problems unless renal function is impaired.

The management of complications occurring after urinary diversion, such as anastomotic leaks, has changed during the past decade, with such advances as the ability to perform percutaneous nephrostomy and the antegrade passage of ureteral catheters through the ureterointestinal anastomosis and into the intestinal segment. If a urinary leak is suspected, this approach is preferred, with the initial hope for spontaneous closure. Excretory urograms and radiographs of the bowel loop provide useful information concerning the status of the urinary conduit. When conservative measures fail or if the conduit is nonviable, revision must be considered.

In our series, eight patients had fistulas, either ureteral or conduit in origin. When fistula occurred in the earlier years of this study, adequate drainage was obtained through the perineal space. Spontaneous healing occurred in two patients. Two patients died of sepsis related in part to the urinary fistula but also to an associated gastrointestinal fistula. The remaining four patients received operative intervention. In a more recent case, temporary diversion with bilateral percutaneous nephrostomies was done; however, this patient eventually required total revision of the urinary conduit. Reoperation for urinary leaks exposes the patient to increased risks, including an increased mortality rate. To avoid transient ureteral obstruction and to protect the ureterointestinal anastomosis, in recent years we have commonly used ureteral stents at the time of conduit construction.

The management of stomal necrosis and retraction varies, depending on the extent of the necrotic process, the presence of viable proximal conduit, and the degree of conduit retraction. Minor or superficial stomal necrosis with minimal retraction can be managed expect-

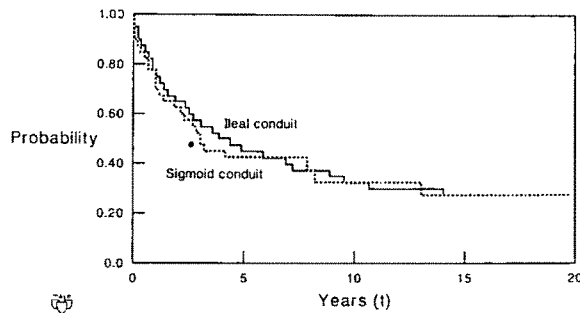


Fig. 1. Kaplan-Meier survival curve of patients with ileal conduit or sigmoid conduit for urinary diversion. Survival was from 30 days after operation.

tantly. Major stomal necrosis down to or deep to fascial planes presents the potential of intraperitoneal retraction and requires prompt revision or, in the worst possible situation, total replacement of the urinary conduit. In one unique situation in which the distal sigmoid urinary conduit underwent necrosis and retraction deep into the panniculus in a very obese patient, a new ileal conduit was isolated and anastomosed to the viable portion of the sigmoid conduit and brought to the skin. This situation emphasized the need to carefully evaluate each patient in whom stomal necrosis occurs and to individualize the treatment based on the findings, the patient's preexisting conditions, and the patient's prognosis.

In conclusion, no significant differences in conduit morbidity or patient mortality were found when ileal and sigmoid conduit urinary diversion procedures were compared. The ileal conduit is the choice after anterior exenteration, whereas the sigmoid conduit is preferable after total exenteration. Conduit of the transverse colon has not been used in the present series but has been

used by us when both the ileum and the colon have been heavily irradiated or are diseased, and this type of urinary diversion is recognized as suitable and seemingly free of complications. Surgical advances to develop an acceptable continent conduit are awaited.

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Pregnancy outcome in patients with insulin-dependent diabetes mellitus with preconceptional diabetic control: A comparative study

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Forty-four of 75 pregnant women with juvenile-onset insulin-dependent diabetes, who attended a preconceptional clinic, were seen regularly by a diabetologic team. Glycemic control was obtained by intensified insulin therapy and monitored by blood glucose self-monitoring. When these patients were compared with a group of 31 nonattenders of the preconceptional clinic, in the former normoglycemia and normal hemoglobin A_{1c} values were achieved before conception, whereas in the latter good control was reached by the second trimester. This group had also more maternal complications, such as preeclampsia, and higher cesarean section rates. Congenital anomalies were 9.6% among offspring of nonattenders, while none occurred in those with preconceptional counseling. We confirm the evidence accumulated in the recent literature that congenital malformations in pregnancy complicated by diabetes may be linked to disturbances in maternal metabolism during the period of embryogenesis. Consequently we concur with the recommendation that tight diabetic control is required before the patient attempts to conceive. (*Am J Obstet Gynecol* 1986;155:293-7.)

Key words: Diabetes in pregnancy, preconceptional diabetic control; pregnancy planning in diabetics

An overall improvement in the perinatal outcome of well-managed diabetic pregnancies has been documented.¹⁻⁴ Pregnancies complicated by diabetes mellitus continue nevertheless to present a significant risk for congenital malformations and neonatal morbidity.^{1, 4-7} Recognition of this risk has led obstetricians to emphasize the need for careful control of maternal diabetes during fetal organogenesis as well as for the entire duration of the pregnancy.^{2, 4, 8-11}

The insult causing malformations in infants of diabetic mothers occurs before the seventh week of gestation.^{4, 12} If strict metabolic control and better management of maternal diabetes may reduce the occurrence of congenital malformations, they must obviously be instituted during this early critical period. Furthermore, careful monitoring of all diabetic women of childbearing age is required, including education before pregnancy, early diagnosis of pregnancy, and skillful diabetic management before conception and during

the early weeks of gestation, a period when most women with diabetes are not yet under medical supervision.

The purpose of this study was to try to provide objective evidence of the benefit of such an approach.

Material and methods

Between the years 1981 and 1985, 75 pregnant women with juvenile-onset insulin-dependent diabetes were treated at the High-Risk Obstetrical Outpatient Clinic of the Golda-Meir Medical Center in association with the Institute of Pediatric and Adolescent Endocrinology, Division of Juvenile Diabetes, Beilinson Medical Center in Israel. Forty-four patients who planned pregnancy attended the preconceptional clinic and consulted with a diabetologic team (obstetrician, diabetologist, dietitian, psychologist, social worker, and nurse) until pregnancy occurred. One patient was advised against pregnancy because of severe malignant retinopathy. She was permitted to conceive after laser therapy. Of the 24 patients who attended the clinic at early stages of pregnancy, 11 had unplanned pregnancies. Seven patients did not attend and failed to appear. All of our patients had been delivered of their infants at the time of submission of this report.

The aims of our preconceptional clinic were as follows: (1) to assess individual patients' fitness for pregnancy, with special attention paid to retinopathy and

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Table I. Control of diabetes in 75 pregnancies in women with insulin-dependent diabetes mellitus

	Period	Women with preconception diabetic control (n = 44) (mean $\pm$ SD)	Other women (n = 31) (mean $\pm$ SD)	Normal pregnant women (n = 20) (mean $\pm$ SD)	p*
Hemoglobin A _{1c} (%)	Initial visit	10.68 $\pm$ 0.49	—	6.76 $\pm$ 0.30†	—
	At conception	7.56 $\pm$ 0.34	—	6.63 $\pm$ 0.29†	—
	First trimester	7.39 $\pm$ 0.34	10.42 $\pm$ 0.47	6.38 $\pm$ 0.29	<0.001
	Second trimester	7.21 $\pm$ 0.33	8.13 $\pm$ 0.37	6.43 $\pm$ 0.29	<0.050
	Third trimester	7.14 $\pm$ 0.32	7.44 $\pm$ 0.34	6.48 $\pm$ 0.29	NS
Blood glucose level (mg/100 ml)	Initial visit	174 $\pm$ 10.8	—	96 $\pm$ 5.9†	—
	At conception	117 $\pm$ 7.3	—	95 $\pm$ 5.9†	—
	First trimester	110 $\pm$ 6.9	163 $\pm$ 10.2	91 $\pm$ 5.6	<0.001
	Second trimester	110 $\pm$ 6.5	148 $\pm$ 9.2	94 $\pm$ 5.9	<0.050
	Third trimester	96 $\pm$ 6.0	124 $\pm$ 7.8	98 $\pm$ 6.1	NS
Insulin dose (U/kg/24 hr)	Initial visit	0.60 $\pm$ 0.03	—	—	—
	At conception	0.62 $\pm$ 0.03	—	—	—
	First trimester	0.71 $\pm$ 0.04	0.83 $\pm$ 0.04	—	<0.001
	Second trimester	0.80 $\pm$ 0.04	0.92 $\pm$ 0.05	—	<0.001
	Third trimester	0.95 $\pm$ 0.05	0.96 $\pm$ 0.05	—	NS

*Statistical analysis by Student's *t* test.

†Normal nonpregnant value.

Table II. Maternal information for 75 pregnant women with insulin-dependent diabetes mellitus

Parameter	Preconceptional diabetic control (n = 44)	Others (n = 31)
Maternal age (mean $\pm$ SD) (yr)	24.2 $\pm$ 5.8	21.4 $\pm$ 2.3
Duration of diabetes (mean $\pm$ SD) (yr)	12.9 $\pm$ 5.4	10.4 $\pm$ 4.4
White's classification ¹⁷		
A	—	—
B	8	7
C	17	15
D	14	8
F	1	—
R	4	1
Parity	1.96	1.4
Duration of pregnancy (mean $\pm$ SD) (wk)	39.3 $\pm$ 0.5	36.9 $\pm$ 0.5
Weight gain (mean $\pm$ SD) (kg)	14.4 $\pm$ 3.6	18.7 $\pm$ 5.2
Preeclampsia (%)	9.1	22.5
Cesarean section (%)	22.7	41.9

nephropathy; (2) to offer advice on contraception, thus to ensure that pregnancy was planned; (3) to identify and treat gynecologic problems; (4) to achieve optimal diabetic control before conception; (5) to identify the time of conception; (6) to obtain maximum cooperation from patients and their partners.

In order to achieve optimal diabetic control before conception, an "intensified insulin therapy" strategy was used, based on a daily regimen of short-acting and intermediate-acting insulins, with additional administration of short-acting insulin when required. In 12

selected cases a continuous subcutaneous insulin infusion pump was used. All patients were advised to adhere closely to their strict, prescribed diet. Dietary instructions allowed a total intake comprising 20% to 25% protein, 30% to 35% fat, and 40% to 45% carbohydrates. Emphasis was placed on three main meals that were equal in their content of carbohydrates and four snacks, each containing identical amounts of carbohydrates; moreover, the "simple sugar/starch" ratio was shifted to 10%/90%. The amount of carbohydrates in each meal was adjusted to blood glucose levels obtained before meals. Control of diabetes was monitored by blood glucose self-monitoring six to eight times per day with glucose meters and measurements twice monthly of hemoglobin A_{1c} by cation exchange column chromatography on a microcolumn fast hemoglobin test system (Isolab, Akron, Ohio). The normal range in our laboratory is indicated in Table I.

In order to facilitate the very early diagnosis of pregnancy patients were asked to use basal body temperature charting through each menstrual cycle. A pregnancy test for the β -subunit of human chorionic gonadotropin was performed whenever 4 weeks elapsed since the last menstrual period; if the first test was negative, testing was repeated at weekly intervals while amenorrhea persisted. In patients who came to medical attention later in the first trimester, hemoglobin A_{1c} levels were measured in the assessment of risk of major congenital malformations.

Evaluation of fetal well-being included monthly serial ultrasound imaging for fetal growth or anomalies starting at 8 weeks' gestation,¹² count of daily fetal movements after 20 to 22 weeks' gestation,¹³ and weekly fetal

Table III. Congenital malformations in offspring of mothers with insulin-dependent diabetes mellitus

Case No.	White's classification	Preconceptional diabetic control	Weeks' gestation at initial maternal hemoglobin A _{1c} measurement	Hemoglobin A _{1c} (%)	Malformation
1	C	No	9½	8.8	Hypertrophic atrial septum
2	B	No	10½	9.2	Polydactyly, left hand
3	D	No	12½	10.2	Agenesis, left kidney

Table IV. Neonatal data for 75 infants of mothers with insulin-dependent diabetes mellitus

	Preconceptional diabetic control (n = 44)	Others (n = 31)	p*
Gestational age (mean ± SD) (wk)	39.3 ± 0.5	36.9 ± 0.5	<0.05
Birth weight (mean ± SD) (gm)	3296.2 ± 234.1	3681.4 ± 261.5	<0.05
1 minute Apgar score (mean ± SD)	8.1 ± 0.7	7.1 ± 0.6	<0.001
5 min Apgar score (mean ± SD)	9.2 ± 0.8	9.0 ± 0.8	NS
Hypoglycemia (<30 mg/100 ml) (%)	11.4	25.8	NS
Hypocalcemia (<8 mg/100 ml) (%)	4.5	9.6	NS
Hyperbilirubinemia (>15 mg/100 ml) (%)	22.7	32.2	NS
Respiratory distress syndrome (%)	2.3	12.9	NS

*Statistical analysis by Students' *t* test.

biophysical profile after 28 weeks' gestation.¹¹ Fetal lung maturity was evaluated by means of a lung profile.¹⁵

Timing and route of delivery were individualized according to maternal and fetal conditions.

Results

Table I presents details on the control of diabetes in the 75 pregnant women. The mean hemoglobin A_{1c} level was 10.68% ± 0.49% and the mean blood glucose level was 174 ± 10.8 mg/100 ml in patients attending our clinic before pregnancy. Although these levels were close to the original hemoglobin A_{1c} level of the nonattenders, normoglycemia and normal hemoglobin A_{1c} levels were achieved before conception and were maintained throughout the pregnancy. Moreover, in spite of the process of improvement in the nonattending group, by the first trimester, hemoglobin A_{1c} levels were still elevated. Significantly higher insulin doses (*p* < 0.001) were required by the latter during the first and second trimesters. Moreover, only by the second trimester was the degree of improved diabetic control similar in both groups. Maternal information concerning the 75 diabetic pregnant women is presented in Table II. Nonattenders were younger and of lower parity and had a shorter duration of diabetes. Nevertheless the frequency of preeclampsia and the cesarean delivery rates in these patients were higher than in the attending group before conception.

Table III summarizes congenital malformations detected in the neonatal period. All congenital anomalies

appeared in offspring of nonattending mothers with high initial hemoglobin A_{1c} levels. The incidence of anomalies in this group was 9.6%. There were three infants with the following congenital malformations, identified by ultrasound scan later in pregnancy: hypertrophic atrial septum, polydactyly of the left hand, and agenesis of the left kidney. No malformations occurred among offspring of those with preconceptional counseling and they had normal serial ultrasound scans. Fetal movement records and weekly fetal biophysical profiles were identical in both groups.

Neonatal data are presented in Table IV. Mean gestational age was significantly longer (39.3 ± 0.5 weeks) in attenders in comparison with nonattenders (36.9 ± 0.5 weeks). Infants of nonattending mothers had significantly higher mean birth weights (3681.4 ± 261.5 gm, *p* < 0.05) and significantly depressed Apgar scores at 1 minute (*p* < 0.0001), but at 5 minutes improved scores were achieved in both groups. Hypoglycemia, hypocalcemia, and hyperbilirubinemia were seen in both groups, more frequently in infants of nonattenders; however, they were treated successfully. Respiratory distress syndrome was seen in 12.9% of the infants of diabetic mothers not seen before pregnancy, whereas only one infant developed this complication in the other group.

Comment

Perinatal morbidity and mortality have decreased significantly in the past two decades in pregnancies com-

plicated by diabetes mellitus.^{1-3, 9, 10} Nowadays, as losses from stillbirths, intrapartum asphyxia, respiratory distress syndrome, and serious metabolic disturbances in the neonatal period have diminished, major congenital anomalies emerge as the one single serious threat constituting the major target for further improvement in the management of the pregnant women with diabetes mellitus.^{1, 4, 5, 8-10}

The overall incidence of fetal anomalies in diabetic pregnancies remains 6% to 9% according to several large series,^{3, 5, 10, 11} namely, three to four times that found in the general neonatal population.¹⁻³ A number of etiologic factors have been proposed to explain the increased rate of congenital anomalies in infants of diabetic mothers. These include genetic influences, teratogenic agents, maternal vascular disease, and metabolic effects of maternal diabetes.^{11, 16}

The fact that poor diabetic control and hyperglycemia in early pregnancy are associated with an increased risk of congenital malformations in offspring is well documented.^{3, 4-6} Moreover, since measurement of hemoglobin A₁ provides an integrated retrospective index of diabetic control, an association between high hemoglobin A₁ levels in early pregnancy and congenital anomalies has been suggested.¹¹ This relates some aspects of the disturbed maternal milieu in the causation of malformations in diabetes.¹⁶ As most fetal malformations occur before 7 weeks' gestation,^{1, 12} when most diabetic pregnant women have not yet come to medical attention, it seems that good metabolic control before conception and during the early weeks of pregnancy may serve as a rather important contributory factor to better management of pregnancy in the diabetic woman.

Furthermore, other concerns for the infant of the diabetic mother are size and metabolic status. As programs of normoglycemia have been accepted for pregnancy complicated by diabetes, the observation that maternal glycemic levels affect fetal size has emerged.^{2, 3, 6, 7} In fact, improved prenatal diabetic management and neonatal care have reduced the incidence of neonatal morbidity almost to that of the nondiabetic population.

This series confirms these findings. We found a significantly higher incidence ($p < 0.07$) of congenital malformations in offspring of women without preconceptional diabetic control, who came to our attention at different stages of pregnancy and who had elevated blood glucose and hemoglobin A₁ values at the initial clinic visit. The incidence and type of anomalies in this group are similar to those reported in other series of pregnant diabetic women.^{3, 5, 10, 11, 16} In fact, sonography was performed in these patients at the initial clinic visit (9 to 13 weeks) but failed to detect early delay of fetal growth; nevertheless each malformation was identified

later in pregnancy. The mild type of anomalies in our series serves as a partial explanation of the normal early sonographic imaging and the postponement of exact identification to more advanced stages in pregnancy. Moreover, while only mildly malformed, these fetuses presented normal fetal movement records and fetal biophysical profiles. No anomalies were detected in offspring of women with well-controlled diabetes before and during early stages of pregnancy.

Fetal movement records and biophysical profiles were identical in both groups. The first is performed after the twentieth to twenty-second week while the latter is started after the twenty-eighth week of gestation—periods of time when both groups were well controlled.

Furthermore, our study reveals a relation between preconceptional blood glucose regulation and the degree of improved diabetic control. Attending patients achieved normoglycemia and normal hemoglobin A₁ values before conception and throughout the first trimester, values that were maintained during pregnancy. They required significantly lower insulin doses during the first and second trimesters because with near-normal glycemia less insulin was needed. Nonattending patients achieved normal levels only by the second trimester. It seems that this factor might have played an important role in the causation of more frequent maternal complications and a higher rate of cesarean deliveries in these patients. Furthermore, we must presume that neonatal outcome was affected as well in this group, as mean gestational age was shorter, mean birth weights were higher, and respiratory distress syndrome, hypoglycemia, hypocalcemia, and hyperbilirubinemia were more frequent. Our experience with preconceptional diabetic control is that it contributed to improved management of pregnancy in the diabetic patient by the achievement of better control of diabetes, particularly at the crucial time of conception and throughout the first trimester. Improved motivation was in fact noted, on the part of our patients, toward better diabetic control and close cooperation between those most concerned in the management of the pregnancy.

It seems that the features of our approach led to the successful management and improved results in our series. We therefore consider this approach as an absolute prerequisite for pregnancy planning in diabetic patients.

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Increased risk of endometritis and wound infection after cesarean section in insulin-dependent diabetic women

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To determine if diabetic women have an increased risk for post-cesarean section endometritis and/or wound infection, all insulin-requiring diabetic women who were delivered by cesarean section between 1977 and 1981 were compared with a group of nondiabetic patients delivered by cesarean section. Patients were divided into low-risk or high-risk groups on the basis of labor and ruptured membranes. Compared with control subjects, diabetic patients were at significantly greater risk for postoperative infectious morbidity. Among diabetic patients, risk for postoperative infections was independent of White's classification of diabetes and gestational age at delivery. The increased rate of infection among the diabetic patients suggests that prophylactic antibiotics might be efficacious for insulin-requiring diabetic patients undergoing cesarean section. (*AM J OBSTET GYNECOL* 1986;155:297-300.)

Key words: Diabetes mellitus, pregnancy, cesarean section, endometritis, wound infection

Pregnancy complicated by diabetes mellitus increases the risk to both mother and infant for peripartum morbidity and mortality.¹⁻⁴ Of particular concern has been the relatively high frequency of stillbirths that have

plagued diabetic pregnancies,^{1,2} the cause of which remains uncertain. In the past, in an attempt to prevent intrauterine fetal death while minimizing the risk of hyaline membrane disease, the diabetic woman was electively delivered at a set gestational age determined by White's classification.⁵ More recently, with the advent of techniques to assess fetal lung maturity by the lecithin/sphingomyelin ratio and phosphatidylglycerol, delivery is often planned as soon as fetal lung maturity can be demonstrated. Because of low Bishop scores, the elective cesarean section rate among diabetic pregnan-

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cies is higher than that among a general obstetric population, and the total cesarean section rate exceeds 50% in most series.³⁻⁴

It is often dogmatically stated, although perhaps incorrectly so, that diabetic patients are at an increased risk for infection.⁶ Perhaps this impression stems from the preantibiotic era when it was considered that "the average duration of life in juvenile diabetics was about seven years followed by death from septicemia."⁷ Documented correlations of diabetes mellitus and an increased risk of infection are limited and have most often related to urinary tract^{8,9} and foot⁸ infections, the latter often occurring in individuals with advanced diabetic vascular disease. Compared with the general population, diabetic patients may or may not be at greater risk for pneumonia and candidiasis.⁹ On the other hand, diabetic women have been found to be especially susceptible to unusual infections such as malignant otitis externa, mucormycosis, and emphysematous cholecystitis.^{8,9}

To our knowledge, it has never been established that insulin-dependent diabetic patients are at increased risk for post-cesarean section infection when compared with nondiabetic women. This report examines the relationship of diabetes mellitus to postoperative endometritis and wound infection, as well as the role played by factors such as gestational age, obesity, diabetic control, and White's classification.

Material and methods

A chart review was performed of all insulin-requiring pregnant diabetic women who underwent cesarean section at Vanderbilt University Medical Center during a 5-year period between January 1, 1977, and December 31, 1981, as previously described.¹ To control for possible alterations in practice patterns during the 5-year period, comparison was made with nondiabetic patients who underwent cesarean section randomly and were selected by a case-control method, two for one during each year of the study.

Diabetic and nondiabetic women were classified as either at high risk or at low risk for subsequent post-cesarean section endometritis or wound infection. High-risk status was defined as ruptured membranes or labor (of any duration) before cesarean section, while low-risk status was defined as the absence of both ruptured membranes and labor preceding cesarean section. Patients were also matched for the use of prophylactic antibiotics. Operative procedures in both groups were performed primarily by resident physicians under faculty guidance. Guidelines for the use of therapeutic or prophylactic antibiotics were similar for all patients and applied irrespective of diabetic status.

The hospital record was assessed for the occurrence of postoperative endometritis and/or wound infection.

Postoperative infectious morbidity for both groups was determined with the generally accepted clinical criterion of fever with either uterine tenderness or purulence of wound. Both groups were assessed in a similar fashion without consideration of diabetic status. Those individuals begun preoperatively on a regimen of therapeutic antibiotic treatment for any type of infection are included descriptively in this report but are excluded from the analysis of postoperative infection rates. Similarly all types of infectious morbidity other than endometritis or wound infection are also excluded from the postoperative infection rates.

Diabetic control was assessed by noting the occurrence of gross clinical manifestations of metabolic derangement: diabetic ketoacidosis, neonatal hypoglycemia, or infant macrosomia.

Statistical significance was determined with the χ^2 test or Fisher's exact test. Data are expressed as mean $\pm$ SEM.

Results

During the 5-year period of this study, 83 pregnant diabetic women were identified who underwent cesarean section, four of whom were receiving therapeutic antibiotics at the time of cesarean section. Of the remaining 79, 55 were at low risk for the development of post-cesarean section infectious morbidity (group 1) and 24 were considered to be at high risk (group 2). Among the 158 nondiabetic patients who underwent cesarean section, 110 were at low risk (group 3), while 48 were considered to be at high risk (group 4). In the process of selecting a two-to-one control group, an additional 24 nondiabetic patients were excluded because of preoperative therapeutic antibiotics. The maternal racial distributions were similar in all groups as was gestational age at delivery between the diabetic and the nondiabetic populations (37 ± 2 and 36 ± 6 weeks, respectively). Review of obstetric risk factors for post-cesarean section infection demonstrated comparable distributions in the diabetic and nondiabetic population of length of labor, use of intrauterine pressure catheter, number of pelvic examinations, and the incidence and duration of ruptured membranes.

Five of 55 (9%) low-risk diabetic patients (group 1) developed post-cesarean section endometritis or wound infection, while among high-risk diabetic women (group 2), the incidence was six of 24 (25%) (Table I). Among nondiabetic women, two of 110 (2%) low-risk (group 3) and three of 48 (6%) high-risk (group 4) individuals developed infections. Thus, in both diabetic and nondiabetic patients, high-risk individuals had a somewhat higher rate of post-cesarean section infection. However, among both low- and high-risk individuals, diabetic women were significantly more likely than nondiabetic women to develop post-cesarean sec-

Table I. Number and percentage of women in each group who developed post-cesarean section endometritis and/or wound infection

	Diabetic		Nondiabetic	
	Group 1: Low risk	Group 2: High risk	Group 3: Low risk	Group 4: High risk
Total no.	55	24	110	48
Patients with endometritis and/or wound infection				
No.	5	6	2	3
%	9.1	25.0*	1.8†	6.3‡§

*Group 1 versus group 2, $p = 0.067$.

†Group 1 versus group 3, $p = 0.042$.

‡Group 2 versus group 4, $p = 0.032$.

§Group 3 versus group 4, NS.

tion endometritis and wound infection ($p = 0.042$ and $p = 0.032$, respectively).

Use of prophylactic antibiotics occurred in one low-risk and six high-risk diabetic women, none of whom developed postoperative infection. Two of the low-risk nondiabetic patients received prophylactic antibiotics and neither developed postoperative infection. Among the high-risk nondiabetic women receiving prophylaxis, one of 12 (8%) developed a postoperative infection. Among diabetic women receiving no prophylaxis, the postoperative infection rate was significantly less ($p < 0.05$) in the low-risk groups (5/54, 9%) as opposed to the high-risk group (6/18, 33%). In contrast, among the nondiabetic patients without antibiotics, the occurrence of infection (low risk, 2/108 or 2%; high risk, 2/36 or 6%) was significantly lower than that of the low- and high-risk diabetic groups ($p < 0.02$ for both). Furthermore, although the numbers are small, the rates of postoperative infection for both low-risk and high-risk groups were not significantly different between diabetic and nondiabetic patients receiving prophylaxis.

Among the diabetic women, the occurrence of post-cesarean section infectious morbidity in both high- and low-risk groups was independent of White's classification (Table II). The infection rate was independent of gestational age. None of these infections occurred among the six patients clinically considered to be obese. Infectious morbidity among low-risk diabetic women was independent of diabetic control, as defined by the occurrence of diabetic ketoacidosis, neonatal hypoglycemia, or infant macrosomia. However, high-risk diabetic women with poorer control, evidenced by one or more of these complications, had an increased incidence (6/14) of infection compared with that of high-risk diabetic women with good control (0/10) ($p < 0.05$).

Table II. Incidence of post-cesarean section endometritis and/or wound infection by White's classification of diabetes

White's classification	Risk group	Total No. of patients	Patients with endometritis or wound infection	
			No.	%
B	Low risk	26	3	12
	High risk	12	4	33
C	Low risk	18	1	6
	High risk	7	2	29
D,F,R	Low risk	11	1	9
	High risk	4	0	

Comment

The medical and surgical literature is replete with reports describing the vulnerability of diabetic patients to infection.^{6, 8, 9} While most of these reports involve individuals with urinary tract infections, vasculopathies, or unusual infections, this characterization has often been assumed to apply to all diabetic patients.⁶ Whether diabetes increases the risk for operative site infections among younger individuals in general, and for pregnant diabetic women in particular, has to our knowledge not been examined.

Reported factors that predispose to post-cesarean section infectious morbidity include duration of labor, membrane status, use of internal fetal monitoring, number of vaginal or rectal examinations, patient socioeconomic class, maternal anemia, estimated gestational age, obesity, and surgical skill and experience of the surgeon.¹⁰⁻¹³ In order to eliminate arbitrary, often ill-defined intervals for duration of labor or ruptured membranes, we have grouped patients into low or high risk on the basis of the total absence or presence of either of these two factors. The value of this grouping has recently been demonstrated by Nielsen and Hokegard.¹¹ They reported, in a series of 1319 cesarean sections, that the postoperative infection rate in elective procedures was 4.1%. However, if membranes were ruptured or labor had begun before cesarean section, the postoperative infection rate was increased to 18% to 19% and essentially remained at this constant level throughout the first 16 hours. Subsequently, with a greater duration of ruptured membranes or labor, the rate of infection increased to 34% to 50% and again plateaued.

In our series, among women undergoing cesarean section, the incidence of postoperative endometritis and/or wound infection was higher among diabetic women than among nondiabetic women. In the low-risk groups, diabetic women were five times more likely to develop postoperative infectious morbidity. Among high-risk patients, endometritis or wound infection occurred in 25% of diabetic patients compared with only

6% of nondiabetic women. Thus, among pregnant women, diabetes mellitus does appear to be a predisposing factor to postoperative infectious morbidity.

The grouping of the diabetic patients by White's classification did not demonstrate an increased frequency of infection among the advanced classes. The absence of a correlation between advanced diabetic class and infectious morbidity may indicate that vascular changes are not responsible for the increased rate of infection seen in these diabetic patients or may simply reflect the small number of patients in this study who have advanced diabetes.

Obesity per se has been shown to be associated with an increased risk of postoperative febrile morbidity.¹² However, among the diabetic patients in this study obesity did not appear to be a factor in the development of endometritis or wound infection. Similarly, while it has been suggested that amniotic fluid antibacterial activity is reduced in gestations <36 weeks,¹³ preterm delivery of the diabetic patients did not result in an increased rate of infectious morbidity.

Hypotheses that have been proposed to explain the increased susceptibility of diabetic patients to infection include vasculopathy, which limits blood flow and thus oxygenation to tissue, neuropathy, which prevents the diabetic patient from being aware that tissue damage is occurring, and diminished white blood cell function.¹⁴⁻¹⁶ The latter is most pronounced in those individuals with poor diabetic control and can be reversed by insulin therapy.¹⁶ This is consistent with our finding that high-risk patients with "poorly" controlled diabetes were at a significantly increased risk for developing post-cesarean section infections.

In the literature, reports of the incidence of post-cesarean section infectious morbidity vary considerably, from 13% to 85%.^{10,11} The incidence in our institution described in this report is lower, perhaps because of (1) the socioeconomic class of our patients, (2) careful exclusion of patients with amnionitis or other conditions requiring preoperative therapeutic antibiotics, and (3) deliberate exclusion of patients with other types of infections, such as urinary tract infection.

The general use of perioperative prophylactic antibiotics to minimize the risk of operative site infections in every patient undergoing cesarean section has been criticized as not being cost effective.^{12,13} Most authorities limit their recommendations for the use of prophylactic

antibiotics to those patients at increased risk for postoperative infections. In view of the high cesarean section rate in diabetic pregnancies and the findings described in this report, which identify insulin-dependent diabetic women undergoing cesarean section as one of these high-risk groups, we suggest consideration of routinely administering prophylactic antibiotics to these women.

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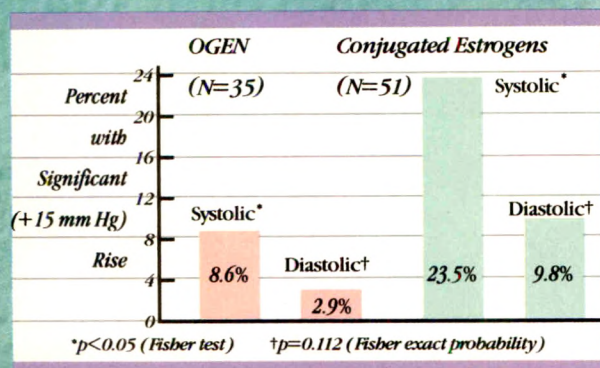
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A complete list of references is available on request.

Her estrogen therapy shouldn't affect her blood pressure



Percent of patients showing BP change of 15 mm Hg or greater after 7.4 months to 7.9 months therapy with either conjugated estrogens or OGEN® (estropipate)



Adapted from Wren et al, 1981!

OGEN dosages ranged from 0.625 to 2.5 daily, 24 out of 30 days. Conjugated estrogen dosages ranged from 0.3 to 2.5, 24 out of 30 days. The majority of those experiencing a change, like the majority of the total sample, were also taking a progestogen, levonorgestrel 0.03 mg, from the 15th to the 24th day.

Because the risk of hypertension increases with age, it is appropriate to examine the effects of estrogen replacement on blood pressure.

"More women receiving...[conjugated estrogens] had a clinically significant rise in blood pressure than those receiving Ogen..."¹

OGEN®
(estropipate)

Only estrone. Identical to the body's own.

*Scored tablets make
precise dosing easy*



OGEN .625

(estropipate 0.75 mg, calculated as
sodium estrone sulfate 0.625 mg)



OGEN 1.25

(estropipate 1.5 mg, calculated as
sodium estrone sulfate 1.25 mg)



OGEN 2.5

(estropipate 3 mg, calculated as
sodium estrone sulfate 2.5 mg)



OGEN 5

(estropipate 6 mg, calculated as
sodium estrone sulfate 5 mg)

■ OGEN tablets are scored so you can adjust dosage without writing a new prescription. It's another reason more physicians are prescribing OGEN.

See adjacent page for brief summary of prescribing information.

Reference:

1. Wren BG, Routledge DA: Blood pressure changes: Oestrogens in climacteric women. *Med J Aust* 2:528-531, 1981.

OGEN
(estropipate)

Only estrone. Identical to the body's own



OGEN® ESTROPIRATE TABLETS, USP Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe vasomotor symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)

2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIRATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. **Induction of malignant neoplasms.** Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examination should be performed periodically.

2. **Gall bladder disease.** A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. **Effects similar to those caused by estrogen-progestogen oral contraceptives.** There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would

be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thromboses in women receiving estrogens for postpartum breast engorgement.

a. **Thromboembolic disease.** It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. **Hepatic adenoma.** Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. **Elevated blood pressure.** Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. **Glucose tolerance.** A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

e. **Hypercalcemia.** Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. **Fluid retention** — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. **Information for the Patient.** See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. **Drug Interactions.** The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

D. **Use of broad spectrum antibiotics** which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving insulin may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

- Increased sulfobromophthalen retention.
- Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
- Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
- Abnormal glucose tolerance test results.
- Decreased pregnandiol excretion.
- Reduced response to metoprolol test.
- Reduced serum folate concentration.
- Increased serum triglyceride and phospholipid concentration.

D. **Carcinogenesis.** Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. **Pregnancy.** Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. **Nursing Mothers.** Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. **Pediatric Use.** Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. **Genitourinary system.**
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Cystitis-like syndrome.
Dysmenorrhea.
Amenorrhea during and after treatment.
Change in cervical eversion and in degree of cervical secretion.
Breakthrough bleeding, spotting; change in menstrual flow.
Premenstrual-like syndrome.
2. **Breast.**
Tenderness, enlargement, secretion.
3. **Gastrointestinal.**
Cholestatic jaundice.
Vomiting, nausea.
Abdominal cramps, bloating.
4. **Skin.**
Hemorrhagic eruption.
Erythema nodosum.
Erythema multiforme.
Hirsutism.
Chloasma or melasma which may persist when drug is discontinued.
Loss of scalp hair.
5. **Eyes.**
Steepening of corneal curvature.
Intolerance to contact lenses.
6. **CNS.**
Chorea.
Mental depression.
Migraine, dizziness, headache.
7. **Miscellaneous.**
Aggravation of porphyria.
Edema.
Reduced carbohydrate tolerance.
Increase or decrease in weight.
Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. Given cyclically for short-term use:

For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms — One OGEN 625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN 625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. Given cyclically:

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control. Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN 625 (0.75 mg estropipate), yellow tablets, NDC 0074-3943-04; OGEN 125 (1.5 mg estropipate), peach-colored tablets, NDC 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, NDC 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, NDC 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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Outstanding mixed aerobic/anaerobic coverage

Cefizox[®] $\frac{IM}{IV}$

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ceftizoxime sodium

Injection: 1 and 2 gram vials

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BELOW THE DIAPHRAGM
including the septic pelvis*

- B.I.D. Dosing[†] for All but Life-Threatening Infections

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A recent, prospective, randomized, comparative trial demonstrated that 'Cefizox' was equal to clindamycin/tobramycin in the treatment of intra-abdominal infections.

- Better Gram-negative and *Bacteroides* Coverage Than Mefoxin[®] (cefoxitin)[‡]

Aerobic Organisms ² (number of strains)	CEFIZOX [®] (ceftizoxime) MIC ₉₀	MEFOXIN [®] (cefoxitin) MIC ₉₀
Gram-Negative	mcg./ml.	mcg./ml.
<i>Escherichia coli</i> (40)	0.04	6.3
<i>Klebsiella pneumoniae</i> (32)	0.05	25
<i>Enterobacter aerogenes</i> (16)	0.12	>400
<i>Enterobacter cloacae</i> (16)	0.8	400
<i>Proteus</i> sp. (indole positive) (26)	6.3	12.5
<i>Citrobacter</i> sp. (32)	0.2	400
Gram-Positive	mcg./ml.	mcg./ml.
<i>Staphylococcus aureus</i> (16)	3.1	3.1

<i>Bacteroides</i> species ³ (number of strains)	CEFIZOX [®] (ceftizoxime) MIC ₉₀	MEFOXIN [®] (cefoxitin) MIC ₉₀
<i>B. fragilis</i> (55)	8 mcg./ml.	16 mcg./ml.
<i>B. ovatus</i> (17)	16 mcg./ml.	64 mcg./ml.
<i>B. thetaiotaomicron</i> (13)	64 mcg./ml.	32 mcg./ml.
<i>B. distasonis</i> (12)	8 mcg./ml.	32 mcg./ml.
<i>B. vulgatus</i> (3)	8 mcg./ml.	16 mcg./ml.

* Due to susceptible strains of indicated organisms.

[†] Dosage in renally compromised patients may require adjustment. Please see complete prescribing information.

[‡] *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Harding, G.K.M., et al.: Rev. Infect. Dis. 6(Suppl. 1) S283-S292, 1984.

2. Fu, K.P., and Neu, H.C.: Antimicrob. Agents Chemother. 17:583-590, 1980.

3. Aldridge, K.E., et al.: Antimicrob. Agents Chemother. 26:410-413, 1984.

Please consult last page of this advertisement for brief summary of prescribing information.

Cefizox[®]

brand of
sterile ceftizoxime sodium
and ceftizoxime sodium injection

Before prescribing, see complete prescribing information in SK&F literature or PDR. The following is a brief summary.

INDICATIONS

'Cefizox' is indicated in the treatment of infections due to susceptible strains of the microorganisms listed below:

LOWER RESPIRATORY TRACT INFECTIONS caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Klebsiella* sp.; *Proteus mirabilis*; *Escherichia coli*; *Haemophilus influenzae* including ampicillin-resistant strains; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Serratia* sp.; *Enterobacter* sp.; and *Bacteroides* sp.

URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli*; *Pseudomonas* sp. including *Ps. aeruginosa*; *Proteus mirabilis*; *P. vulgaris*; *Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp. including *S. marcescens*; *Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae*.

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli*; *Staphylococcus epidermidis*; *Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis*; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli*; *Bacteroides* sp. including *B. fragilis*; *Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis*; *Escherichia coli*; *Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis*; *Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis*; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis*; *Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae*. 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae*.

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use. Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox', nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatinine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the most frequent adverse reactions (greater than 1%, but less than 5%) are: **Hypersensitivity**—Rash, pruritus, fever. **Hepatic**—Transient elevation in SGOT, SGPT and alkaline phosphatase. **Hematologic**—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. **Local**—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (less than 1%) are: **Hypersensitivity**—Numbness has been reported rarely. **Hepatic**—Elevation of bilirubin has been reported rarely. **Renal**—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. **Hematologic**—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. **Genitourinary**—Vaginitis has rarely occurred. **Gastrointestinal**—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in 'Piggyback' Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

BRS — CF:L14

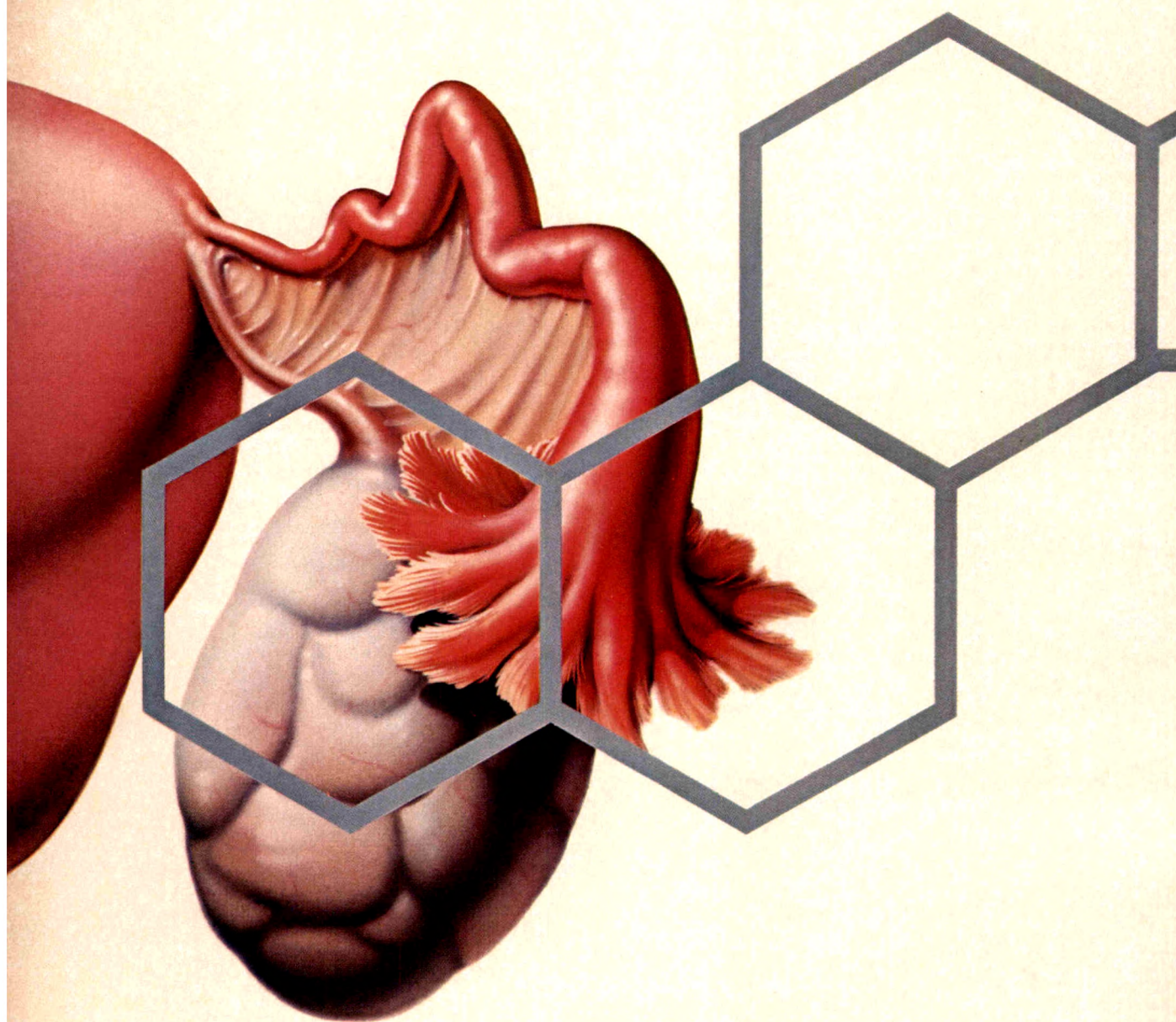
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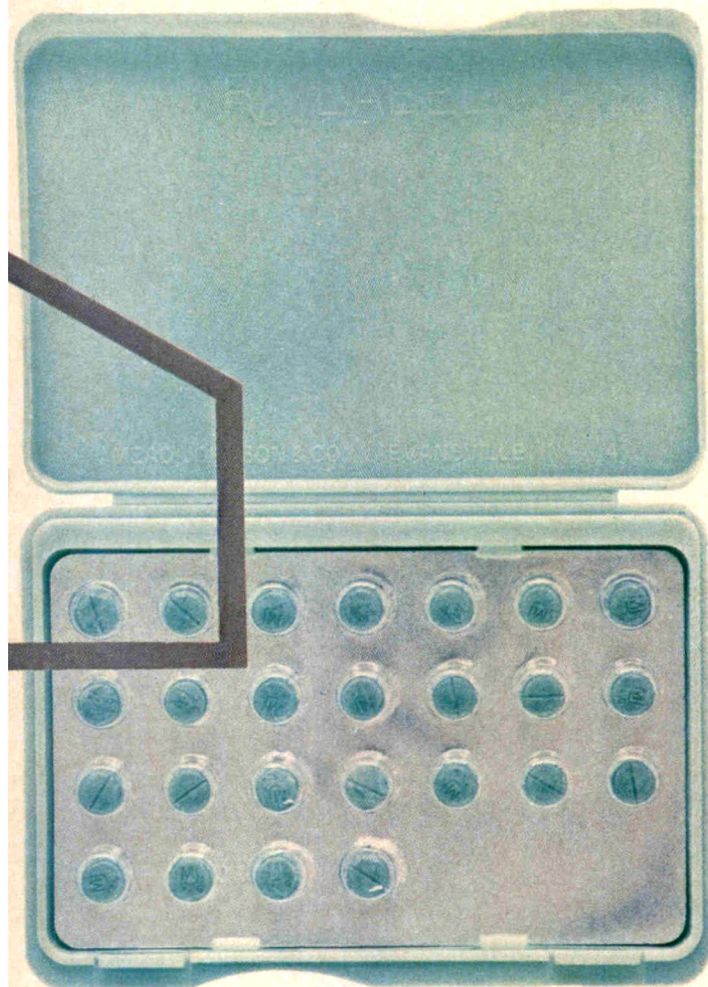
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THE RIGHT
REPLACEMENT
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 17β -ESTRADIOL



THE RIGHT REGIMEN

THE FIRST
25 DAYS OF
THE MONTH—
EVERY MONTH



ESTRACE is 17β -estradiol—just like the estrogen she made before menopause. Like the natural hormone, it follows a simple, well-known metabolic pathway, and it provides unsurpassed relief of postmenopausal vasomotor symptoms.

Micronized for good oral absorption, ESTRACE produces prompt, predictable serum levels of estradiol and estrone. Between cycles, ESTRACE is rapidly eliminated, providing estrogen replacement therapy that closely follows the premenopausal pattern... *for true cyclic therapy.*

Now it's in the ESTRACE Cycle Pak—an easy-to-carry compact, designed to deliver a starting dose of 2 mg in the preferred 25-day regimen. Cycle Paks delivering 1 mg daily are also available, if needed.

MeadJohnson LABORATORIES

For Brief Summary, please see following page.

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1 mg and 2 mg
CYCLE PAK
HAS BOTH

ESTRACE® (estradiol)

TABLETS, 1 mg and 2 mg VAGINAL CREAM, 0.01%

Warning

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY. The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe vasomotor symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma—palliative therapy of advanced disease. *Vaginal Cream:* Atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or

abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc., may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnandiol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration.

Information for the Patient—See text of Patient Package Insert.

Pregnancy Category X—See Contraindications and Boxed Warning.

Nursing Mothers—Administer to nursing mothers only when clearly necessary.

The 2-mg tablets contain FD&C Yellow No. 5 (tartrazine), which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, frequently those who also have aspirin hypersensitivity.

Adverse Reactions: (See Warnings regarding induction of neoplasia, adverse effects on the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported: *Genitourinary system*—Breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical eversion and in degree of cervical secretion; cystitis-like syndrome. *Breast*—Tenderness, enlargement, secretion. *Gastrointestinal*—Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice. *Skin*—Chloasma or melasma which may persist when drug is discontinued; erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism. *Eyes*—Steepening of corneal curvature; intolerance to contact lenses. *CNS*—Headache, migraine, dizziness; mental depression; chorea. *Miscellaneous*—Increase or decrease in weight, reduced carbohydrate tolerance, aggravation of porphyria, edema, changes in libido.

How Supplied: Tablets, containing 1 mg (lavender) and 2 mg (turquoise) micronized estradiol, in bottles of 100 and compacts of 25; Vaginal Cream, each gram containing 0.1 mg 17 β -estradiol in a nonaqueous base containing purified water, propylene glycol, stearyl alcohol, white ceresin wax, glyceryl monostearate, hydroxypropyl methylcellulose, sodium lauryl sulfate, methylparaben, disodium edetate, and *t*-butylhydroquinone, in 1½-oz (42.5-g) tubes with calibrated plastic applicator for delivery of 1, 2, 3, or 4 g and in 1½-oz (42.5-g) refill tubes.

For Prescribing Information, please see physician package insert.

Mead Johnson LABORATORIES
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MJL 5-4169
C-V14-12-R5

Miles Pharmaceuticals Announces...

New **Mycelex[®] Twin Pack** (clotrimazole)

One-day therapy

Mycelex[®]-G 500 mg
(clotrimazole) Vaginal Tablet

**The shortest regimen
for vulvovaginal
candidiasis**

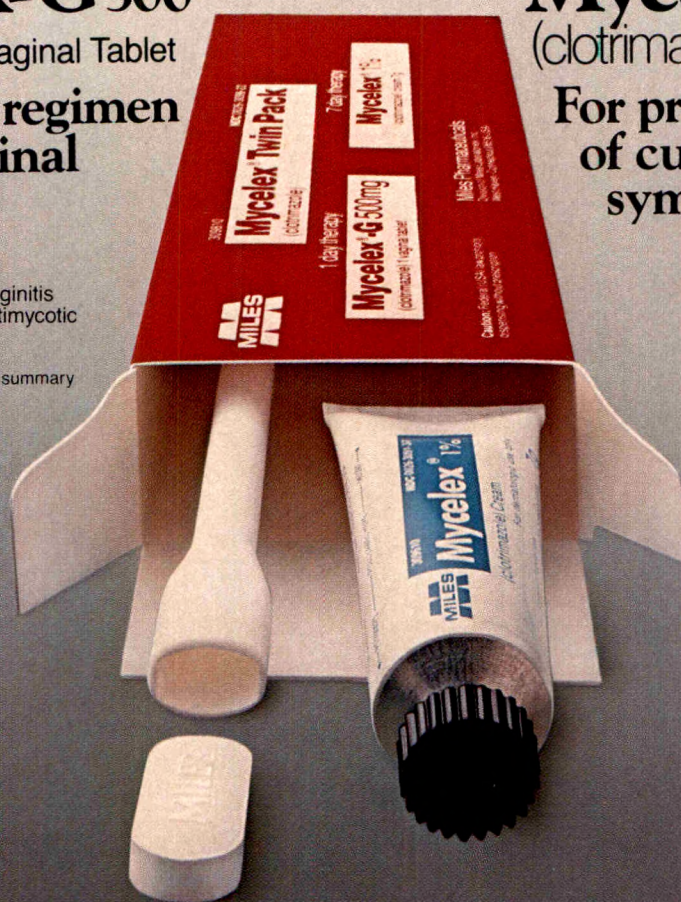
In the case of severe vulvovaginitis
due to candidiasis, longer antimycotic
therapy is recommended.

Please see following page for brief summary
of prescribing information.

Seven-day therapy

Mycelex[®] 1%
(clotrimazole) Cream

**For prompt relief
of cutaneous
symptoms**



Mycelex[®] Twin Pack

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MILES
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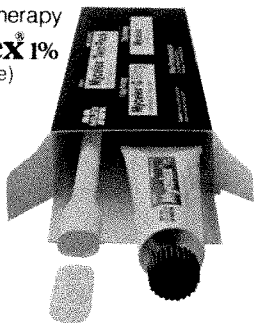
Mycelex® Twin Pack (clotrimazole)

One-day therapy

Mycelex-G 500^{mg}
(clotrimazole) Vaginal Tablet

Seven-day therapy

Mycelex 1%
(clotrimazole)
Cream



Indications

MYCELEX-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one-day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.

MYCELEX-G CREAM is indicated for the topical treatment of dermal infections due to *Candida albicans*.

Contraindications

MYCELEX-G 500 mg Vaginal Tablets and MYCELEX CREAM are contraindicated in women who have shown hypersensitivity to any of their components.

Warnings

MYCELEX-G 500 mg Vaginal Tablets: none. MYCELEX CREAM is not for ophthalmic use.

Precautions

MYCELEX-G 500 mg Vaginal Tablets: If there is a lack of response to the Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

MYCELEX CREAM: If irritation or sensitivity develops with the use of Mycelex Cream, treatment should be discontinued and appropriate therapy instituted.

Carcinogenesis

MYCELEX-G 500 mg Vaginal Tablets: No long-term studies in animals have been performed to evaluate the carcinogenic potential of 500 mg Vaginal Tablets intravaginally. A long-term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

Usage in Pregnancy: Pregnancy Category B:

MYCELEX-G 500 mg Vaginal Tablets: The disposition of ¹⁴C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

MYCELEX CREAM: In the first trimester of pregnancy, Mycelex Cream should be used only when considered essential to the welfare of the patient.

Adverse Reactions

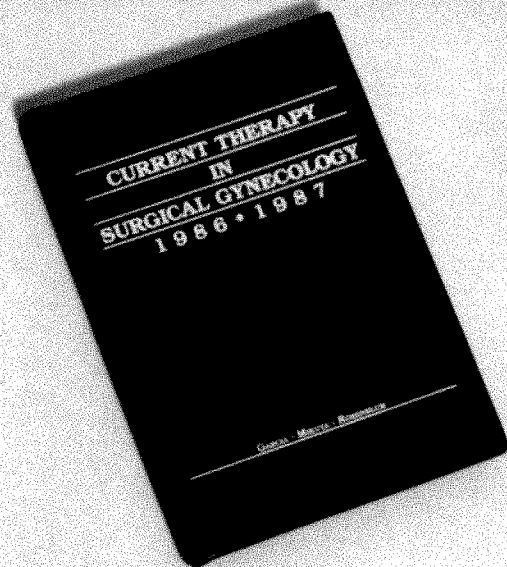
MYCELEX-G 500 mg Vaginal Tablets: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

MYCELEX CREAM: The following reactions have been reported in connection with the use of this cream: erythema, stinging, blistering, peeling, edema, pruritus, urticaria, and general irritation of the skin.

Issued, July 1986

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AMS-939-031-03

Student response to gynecologic teaching associates

Charles R. B. Beckmann, M.D., M.H.P.E., Barbara F. Sharf, Ph.D.,
Barbara M. Barzansky, Ph.D., M.H.P.E., and William N. Spellacy, M.D.

Chicago, Illinois

Gynecologic teaching associates teach the communication and psychomotor skills for breast and pelvic examinations in most medical schools in the United States and Canada. Evaluations of these gynecologic teaching associate programs have included faculty impressions of program effectiveness and measurement of student retention of skills but not student evaluations of the effectiveness and value of such programs. At the University of Illinois at Chicago students evaluated their gynecologic teaching associate program. High ratings were given to program organization and content; the knowledge, ability, and professionalism of the teaching associates; and the outcomes of the sessions with respect to the learning skills needed to perform the examinations. Students emphasized the importance of the ability of the teaching associates to provide immediate informative feedback and to reduce anxiety during the teaching sessions. Students tended to view their ability to perform breast examination to be greater than for the pelvic examinations but felt they learned more about both in the sessions. This information supports the value of the gynecologic teaching associate model in medical education. (AM J OBSTET GYNECOL 1986;155:301-6.)

Key words: Gynecologic teaching associates, breast examination, pelvic examination

Gynecologic teaching associates are nonphysician women specially trained to teach the communication and psychomotor skills needed for obtaining the obstetric-gynecologic history and performing breast and pelvic examinations in a format wherein they provide immediate instruction and feedback to the student while themselves being examined. This instructional method, building on the work of Kretzschmar and Guthrie,^{1,2} and Guenther et al.,³ is widely used in the United States and Canada. Ninety-three percent of medical schools use either the gynecologic teaching associate model of instruction or a slight modification thereof.⁴

At the University of Illinois at Chicago a program using the gynecologic teaching associate model was initiated in the 1983-1984 academic year. The goal is to provide instruction in the psychomotor and communication skills involved in the woman's health history and the breast and pelvic examinations. The program is offered in the physical diagnosis course of the second year and again in the required third-year clerkship in obstetrics and gynecology. An increasing percent of students in the second and third years are being taught in this program. By 1986 all students will participate

in teaching sessions in their second year and again in their third year of medical school.

Evaluations of gynecologic teaching associate programs typically included subjective faculty members' impressions of program effectiveness, measurements of student retention of psychomotor and communication skills, and descriptions of the cost effectiveness of the programs.¹⁻⁶ Subjective student responses have indicated overall satisfaction with such programs. Students have generally not been asked to evaluate specific program components or to provide judgments about how they have benefited. However, this more detailed information about student perceptions of how the experience contributed to their learning would be helpful to educational program planners. This report describes the reactions of second- and third-year medical students to each component of their experience. Data from the first 2 years of the gynecologic teaching associate program at the University of Illinois at Chicago are included.

Structure of the teaching associates program

The program begins with didactic lecture-discussion sessions (two for the second-year students in the physical diagnosis course and one condensed version for the third-year students in the basic clerkship in obstetrics and gynecology) led by the gynecologist program director and consultants from the Center for Educational Development. These sessions dealt with the communication and psychomotor skills required for the comprehensive woman's health evaluation. Instructional techniques include didactic lectures, dis-

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Table I. Second-year students: Evaluation of teaching sessions (N = 173)

Item	Rating				
	1	2	3	4	5
Lecture 1: The physical examination (21 = no response) (No.)		2	31	67	52
Lecture 2: Communication skills (4 = no response) (No.)		3	14	72	80
Film: <i>I Hate This</i> (21 = no response) (No.)	1	6	33	86	26
How would you rate the information provided? (No.)			3	53	117
How well was the session designed to alleviate anxiety? (No.)			3	37	133
How useful was the teaching session? (1 = no response) (No.)			2	23	147

Rater scale: 1 = Unacceptable; 5 = outstanding.

Table II. Second-year students: Comparison of student evaluation of how much they learned with their competence in pelvic and breast examinations (N = 173)

Item	Rating				
	1	2	3	4	5
Pelvic examination					
How much do you feel you learned about the pelvic examination? (2 = no response)					
No.			3	53	110
%			5	31	64
How much do you feel that you learned about communication during the pelvic examination? (1 = no response)					
No.		1	3	44	119
%		1	5	26	69
How would you rate your competence in performing the pelvic examination? (2 = no response)					
No.	2	7	81	63	18
%	1	4	47	37	11
Breast examination					
How much do you feel you learned about the breast examination? (2 = no response)					
No.			45	82	44
%			26	48	26
How much do you feel that you learned about communication during the breast examination?					
No.	3	57	65	78	
%	2	16	38	45	
How would you rate your competence in performing the breast examination? (3 = no response)					
No.	5	72	64	29	
%	3	42	38	17	

Rater scale: 1 = Unacceptable; 5 = outstanding.

cussion sessions, role playing, and a film demonstrating the proper performance of the psychomotor skills of the pelvic examination. In addition, the film *I Hate This*, produced at the University of Tennessee, is used to facilitate discussion of issues of communication, sexuality, and sexism related to the examinations. The film, which deals with the attitudes and emotional reactions of a patient and examiner during a pelvic examination, allows the audience to "hear" the unstated thoughts of examiner and patient while a routine and technically well-done pelvic examination is performed.⁷ Apart from these lecture-discussion sessions, instruction is conducted entirely by gynecologic teaching associates.

Students meet in groups of six with two teaching

associates for an initial introduction and discussion, after which three students from each group meet with the gynecologist program director for a didactic session about the content of the woman's health history. The other three students work with the teaching associates. One teaching associate acts as the patient-teaching associate and is examined, while the other is the instructor-teaching associate, taking the place traditionally filled by a physician-instructor. Both teaching associates, however, provided instruction and feedback. After the three students each spend about 30 minutes in practice, the teaching associates exchange roles and the two groups of three students switch places, the sequence then being repeated. At the end of the sessions, teaching associates complete evaluations of the stu-

Table III. Third-year students: Evaluation of teaching sessions by two groups of students—those with no previous teaching associate experience and those with experience with teaching associates functioning as gynecologic teaching associates (N = 119)

Item	Rating				
	1	2	3	4	5
Lecture 1: The physical examination (24 = no response) (No.)					
No teaching associate experience			2	15	39
Previous teaching associate experience			1	14	24
Lecture 2: Communication skills (25 = no response) (No.)					
No teaching associate experience			2	13	40
Previous teaching associate experience			1	13	25
Film: <i>I Hate This</i> (32 = no response) (No.)					
No teaching associate experience		3	8	28	13
Previous teaching associate experience	1	1	1	22	10
How would you rate the information provided? (No.)					
No teaching associate experience				23	48
Previous teaching associate experience				8	40
How well was the session designed to alleviate anxiety? (No.)					
No teaching associate experience		1	3	10	57
Previous teaching associate experience			1	4	43
How useful was the teaching session? (No.)					
No teaching associate experience			1	10	60
Previous teaching associate experience				3	45

Rater scale: 1 = Unacceptable; 5 = outstanding.

dents' performance. Grading is on a pass-fail basis. At the same time, students complete a questionnaire about the program.

Methodology

The questionnaire given to the students asks that they respond to questions or statements about the program using a five-point rating scale in which 1 is unacceptable and 5 is outstanding. There are three groups of questions with a space for student comments after each section.

The first group of questions deals with the teaching sessions, first asking a student's opinion of the two didactic lecture sessions dealing with communication and psychomotor skills and of the film *I Hate This*. Students are also asked about the content of the sessions, how well the sessions alleviated student anxiety, and the overall usefulness of the sessions.

The second group of questions asks for evaluations of the instructor-teaching associate and patient-teaching associate in three areas: knowledge, ability to provide clear instructions and feedback, and ability to be supportive.

The third group of items asks for the students' evaluation of the outcome of their instruction in both the breast and pelvic examinations in three areas: overall learning during the session, learning about communication skills during the sessions, and perceived competence in the procedure.

In addition, third-year students are asked to indicate whether they have had previous instruction in women's

health evaluation. If they have, they are asked whether the instruction was in a community hospital affiliate of the University of Illinois or in a branch of University Hospital and whether teaching associates were involved in the instruction and also to compare the quality of the program just completed with their previous learning experience. A five-point rating scale is provided where 1 indicates a quality "much lower today" and 5 a quality "much greater today."

Results

Second-year students. All 173 participating second-year students completed questionnaires. Most of these responded to all the components of the first question group dealing with the teaching sessions (see Table I). Seventy-eight percent of responding students rated the lecture-discussion on the physical examination as good or outstanding and 90% rated the lecture-discussion on communication skills as good or outstanding, while 74% rated the film *I Hate This* as good or outstanding. Ninety-eight percent of the 173 students rated the teaching sessions as good or outstanding with respect to information provided and session design to alleviate student anxiety. Ninety-nine percent of responding students indicated the sessions to be good or outstanding with respect to usefulness.

An average of 84% of students rated both the patient-teaching associate and instructor-teaching associate as outstanding with respect to knowledge, ability to provide clear instruction and feedback, and ability to be supportive. Ninety-seven percent of all students

Table IVA. Third-year students: Evaluation of outcome of instruction in pelvic examination by two groups of students—those with no previous teaching associate experience and those with experience with teaching associates functioning as gynecologic teaching associates (N = 119)

Item	Rating				
	1	2	3	4	5
Pelvic examination					
How much do you feel you learned about the pelvic examination?					
No teaching associate experience					
No.			6	26	41
%			5	22	34
Previous teaching associate experience					
No.			1	18	28
%			1	15	24
How much do you feel that you learned about communication during the pelvic examination?					
No teaching associate experience					
No.			5	25	42
%			4	21	35
Previous teaching associate experience					
No.			1	10	36
%			1	8	30
How would you rate your competence in performing the pelvic examination?					
No teaching associate experience					
No.		3	27	33	9
%		3	23	28	8
Previous teaching associate experience					
No.		2	14	26	5
%		2	12	22	4

Rater scale: 1 = Unacceptable; 5 = outstanding.

rated both the patient- and instructor-teaching associates as good or outstanding in these three areas.

Students gave high ratings to the outcome of the sessions with respect to how much they learned (see Table II). Ninety-five percent of students rated how much they learned about the pelvic examination and communication skills during the pelvic examination as good or outstanding; similarly, 74% and 83% of students rated the same respective parameters about the breast examination as good or outstanding. In contrast, 48% of students rated their competence in the pelvic examination as good or outstanding compared with 55% for the breast examination. Forty-one students provided written comments about their experience, eight students remarking on the importance of communication, especially with respect to attention to patient comfort and cooperation. Five students made particular reference to the abilities of the gynecologic teaching associates to reduce student anxiety during the teaching sessions.

Third-year students. Varying numbers of students responded to the questions in the first question group dealing with preliminary didactic instruction, in part because these sessions were not offered to one group. Ninety-seven percent of responding students evaluated the lecture-discussion session as good or outstanding. Eighty-four percent of responding students rated the

film *I Hate This* as good or outstanding. All 119 students responded to the last three questions of the first question group. All students rated the information provided as good or outstanding, 96% rated the session design to alleviate anxiety as good or outstanding, and 99% rated the usefulness of the sessions as good or outstanding. These same patterns of responses were seen from students with and without previous gynecologic teaching associate experiences (see Table III).

With one exception, all students rated the patient-teaching associate and instructor-teaching associate as outstanding or good with respect to knowledge, ability to provide clear instruction-feedback, and ability to be supportive. One student rated his instructor-teaching associate as outstanding but his patient-teaching associate as average. As in the first question group, outstanding ratings predominated and there was no difference on the basis of previous gynecologic teaching associate contact.

The predominance of outstanding and good ratings was also seen in the third question group concerning how much students felt they learned about the breast and pelvic examinations and about communication skills for these examinations (see Tables IVA and IVB). Students differed in their evaluation of their own abilities. Twelve percent of students rated themselves as outstanding in the pelvic examination compared with

Table IVB. Third-year students: Evaluation of outcome of instruction in breast examination by two groups of students—those with no previous teaching associate experience and those with experience with teaching associates functioning as gynecologic teaching associates (N = 119)

Item	Rating				
	1	2	3	4	5
Breast examination					
How much do you feel you learned about the breast examination?					
No teaching associate experience					
No.		5	10	30	26
%		4	8	25	22
Previous teaching associate experience					
No.		2	6	18	22
%		2	5	15	18
How much do you feel that you learned about communication during the breast examination?					
No teaching associate experience					
No.		3	9	27	32
%		3	8	23	27
Previous teaching associate experience					
No.		1	3	17	27
%		1	3	14	23
How would you rate your competence in performing the breast examination?					
No teaching associate experience					
No.		2	10	32	29
%		2	8	27	24
Previous teaching associate experience					
No.		2	9	26	9
%		2	8	22	8

Rater scale: 1 = Unacceptable; 5 = outstanding.

Table V. Medical student self-evaluation of competence in the breast and pelvic examinations (N = 173 second-year medical students and N = 119 third-year medical students)

Student's self-rating	Breast examination		Pelvic examination	
	Second-year students	Third-year students	Second-year students	Third-year students
Unacceptable (%)	0	0	1	0
Below average (%)	5	3	5	4
Average (%)	42	16	47	34
Good (%)	37	49	37	50
Outstanding (%)	17	32	11	12

32% for the breast examination. About the same percentages, 50% and 49%, respectively, rated themselves as good at both examinations, but 35% rated themselves as average in the pelvic examination whereas 16% judged themselves as average in performance of the breast examination. Forty-one students provided written comments about their experience, most remarking on the importance of practice opportunities involving immediate feedback and the value of the ability of teaching associates to decrease student anxiety.

Third-year students had a slightly better self-evaluation of their competence than second-year students. By the end of the third year students felt somewhat more confident with the breast examination than with the pelvic examination although confidence in both examinations increased (see Table V).

One hundred eighteen of the 119 students rated

their third-year experience against that of their second year. Fifty-six of the students responding had had no previous experience with teaching associates and the remainder had had some instruction involving trained models or actual teaching associates. Eighty-five of the 118 responding students rated the program as outstanding or above average.

Comment

In the first 2 years of its operation, the teaching associates program received high ratings from second-year students in the physical diagnosis course and third-year students rotating on the clerkship in obstetrics and gynecology with respect to the organization and function of the teaching sessions themselves; the knowledge, ability, and professionalism of the teaching associates; and the outcomes of the sessions with respect to knowl-

edge gained about the examinations and the communications skills needed to perform the examinations. Students emphasized the ability of the teaching associates to provide immediate informative feedback and to reduce anxiety during the teaching sessions as important to this evaluation. Students tended to view their ability to perform breast examination to be greater than for the pelvic examinations but felt they learned more about both in the sessions. These trends applied to students with and without previous experiences with gynecologic teaching associates. These detailed data support the value of the gynecologic teaching associate model in instruction in the psychomotor and communication skills involved in women's health evaluation.

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Para-aortic node biopsy in cervical and endometrial cancers: Does it affect survival?

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Two hundred ten patients with endometrial and cervical carcinoma had para-aortic node biopsies. Nineteen of the 210 patients (9.0%) had positive para-aortic nodes. These 19 patients received pelvic irradiation, and 18 patients received para-aortic irradiation. The incidence of para-aortic nodal involvement in cervical carcinoma was directly related to the stage of the disease. Eleven of the 12 patients with cervical carcinoma and positive para-aortic nodes received both pelvic and para-aortic irradiation. Three of these patients are alive without disease, resulting in a survival rate of 25%. These patients are surviving for 16, 30, and 41 months. The incidence of positive para-aortic nodes in endometrial adenocarcinoma was related to the uterine length and the histologic grade. The survival rate for patients with endometrial adenocarcinoma and positive para-aortic nodes in this study was 57.1%. Four patients have survived for 1, 30, 60, and 71 months. There were no surgical deaths or radiation therapy complications directly attributable to para-aortic biopsy or irradiation. (*AM J OBSTET GYNECOL* 1986;155:306-14.)

Key words: Para-aortic nodes, biopsy, survival

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The history of gynecologic oncology indicates a continuous search to determine the extent of pelvic malignancies. One of the first areas of investigation in this search was the pelvic lymph nodes. It is natural that scientific curiosity would eventually determine that the next level of spread was the para-aortic nodes. It is an axiom that the patient cannot be cured if all of the disease is not treated.

Lymphangiography was the earliest of several dif-

Table I. Para-aortic node biopsy

Disease and stages	Patients	
	n	%
Cervix (n = 120)		
IB	56	26.7
IIA	5	2.4
IIB	25	11.9
IIIB	21	10.0
IVA	4	1.9
Recurrent	5	2.4
Unknown	3	1.4
Lymphoma	1	0.5
Endometrium (n = 90)		
IAI	20	9.5
IAII	20	9.5
IAIII	4	1.9
IBI	5	2.4
IBII	8	3.8
IBIII	9	4.3
II	19	9.0
III	3	1.4
IV	1	0.5
Recurrent	1	0.5

ferent methods used to determine the extent of lymphatic spread. A para-aortic node biopsy, for a variety of reasons, is a method under extensive investigation. This paper consists of a retrospective review of 210 patients with endometrial or cervical malignancies, all of whom have had para-aortic node biopsies.

This review was performed to provide information for several frequently raised questions. Does the presence of positive para-aortic nodes result in modification of the patient's therapy? Does the evidence gained from a para-aortic node biopsy improve patient survival?

Material and methods

Two hundred ten patients with endometrial or cervical malignancies had para-aortic node biopsies performed by physicians in the section of gynecologic oncology at St. John's Mercy Medical Center. Table I lists the etiology and stage of the diseases in these patients. All of the para-aortic node biopsies were performed through a transperitoneal incision. All biopsies were performed by third-year residents on the gynecologic oncology rotation under the supervision of one of the senior authors (J. G. B. or K. A. H.).

A routine preoperative evaluation, performed in all patients, consisted of blood studies (complete blood count, platelet count, SMA 6 and 12), intravenous pyelogram, barium enema, cystoscopy, and chest X-ray examination. Only patients with cervical carcinoma had an examination under anesthesia.

These para-aortic node biopsies were performed at various intervals as determined by the treatment protocols. The biopsies were performed routinely in patients with cervical carcinoma during the first cesium application approximately halfway through the exter-

Table II. Patients with positive para-aortic nodes and stage of disease

Disease and stages	Patients	
	n	%
Cervix (n = 120)		
IB	7	5.8
IIB	1	0.8
IIIB	4	3.3
Total	12	10.0
Endometrium (n = 90)		
IAII	1	1.1
IAIII	1	1.1
IBIII	2	2.2
II	2	2.2
III	1	1.1
Total	7	7.8

Table III. Endometrial adenocarcinoma—incidence of positive para-aortic nodes

	% of positive nodes
Stage	
IA	4.5
IB	9.1
Grade	
1	0.0
2	3.6
3	21.3
Stage	
IAI	0.0
IAII	5.0
IAIII	25.0
IBI	0.0
IBII	0.0
IBIII	25.0

nal radiation treatments. The biopsies in patients with endometrial adenocarcinoma coincided with their total abdominal hysterectomy and bilateral salpingo-oophorectomy. All of the patients receiving pelvic and para-aortic external radiation were treated through anterior and posterior ports with a 25 Mv Betatron. These patients received an average of 180 to 200 rad per day to the pelvis or para-aortic area during the treatment time of 3 to 6 weeks. The brachy therapy was administered with use of Fletcher suit tandem, colpostats, and after-loaded Heyman capsules.

Nineteen patients had positive para-aortic nodes, and all received pelvic irradiation. Eighteen of these 19 patients (94.7%) received para-aortic irradiation.

Of the 18 patients who received para-aortic irradiation, 14 patients received between 4000 and 5000 rad, two patients received <4000 rad, and two patients received between 5000 and 5500 rad. The para-aortic fields were 6 to 8 cm in width and 10 to 13 cm in length depending on the individual's height.

Twelve of the 19 patients (63.2%) received between 4500 to 6000 rad to the pelvis. Thirteen of the 19 pa-

Table IV. Stage of cervical carcinoma and incidence of positive para-aortic nodes

Study	Stage IE		Stage II		Stage III		Stage IV	
	n	% positive	n	% positive	n	% positive	n	% positive
Buchsbaum ⁸	—	—	12	8.3	20	35	2	50
Averette et al. ⁵	—	—	18	22	10	20	2	50
Piver et al. ¹¹	—	—	9	0	14	35.7	3	66.6
Nelson et al. ⁷	—	—	31	16.6	28	46.4	—	—

tients (68.4%) received preoperative intracavitary radiation, and eight of these patients (42.1%) received between 4500 to 6000 mg hours. Most of the pelvic irradiation was given through field sizes varying between 15 × 18 and 15 × 14 cm.

Results

Two hundred ten patients with endometrial and cervical malignancies had para-aortic node biopsies performed. Nineteen of the 210 patients (9.0%) had positive para-aortic nodes. Table II lists the histology and stage of the disease in these 19 patients.

The number of positive nodes per patient ranged from one to >10. Twelve patients had one to two positive nodes, three patients had three to four, three patients had five to six, and the number of positive nodes was not recorded in one patient.

There were no operative deaths among these 210 patients. Five of the 210 patients (2.4%) had complications related to the para-aortic node biopsy. Operative injury occurred once to the vena cava, external iliac, and common iliac vein. These were minor and were corrected immediately. Two ureters were nicked, and both were immediately repaired with no complications. There were no small bowel obstructions, retroperitoneal lymphocysts, or hematomas.

Since most patients (17 of 19, 89.5%) had both para-aortic and pelvic irradiation, it is impossible to assign minor complications such as diarrhea, nausea, and vomiting specifically to either treatment area. There have been no deaths or upper gastrointestinal tract complications that could be attributed to para-aortic irradiation, that is, gastric or small bowel ulcers or denervation or obstruction of the small intestine.

Two of the 19 patients with positive para-aortic nodes did not receive full pelvic or para-aortic irradiation. One patient with endometrial adenocarcinoma and one patient with cervical carcinoma and a positive left supraclavicular node and vertebral metastases were treated with adjuvant chemotherapy.

Eleven of the 12 (91.7%) patients with cervical carcinoma and positive para-aortic nodes received both pelvic and para-aortic irradiation. Three of these patients with cervical carcinoma treated with radiation therapy are alive without evidence of disease; eight died of the disease. The patient receiving chemotherapy also

died. The survival rate is 25.0%. The length of survival of these three patients is 16 months, 30 months, and 41 months.

The incidence of positive para-aortic nodes in patients with endometrial adenocarcinoma appears to be directly related to the size of the uterus although this is not statistically significant. The incidence is related to the histologic structure of the disease (Table III). Four patients with endometrial adenocarcinoma are alive without evidence of disease, and three have died of the disease. The survival rate is 57.1%. The length of survival for these patients is <1 month, 30 months, 64 months, and 71 months.

Comment

Lymphangiography is one of the methods that has been used for years to determine metastatic involvement of pelvic and para-aortic lymph nodes. Piver and Barlow¹ list the following reasons for the inaccuracy of lymphangiography: all of the pelvic and para-aortic lymph nodes are not filled and thus do not appear on lymphangiograms, <3 mm of metastatic disease in lymph nodes will not result in a defect large enough to be identified on lymphangiograms, and lymph nodes are frequently completely replaced by metastatic disease and therefore will not opacify and will not be visible on lymphangiography.

Computerized tomographic scans have been used in an attempt to determine the presence of metastatic disease in a variety of carcinomas. Hall et al.² stated that the retroperitoneal space was very difficult to scan. Shaner et al.³ stated that the computerized tomographic scan was not useful for detecting nodal disease resulting from ovarian metastases because the nodes are not enlarged.

Lymph node scans using gold Au 198 have been studied by Littman et al.⁴ They reported that the correlation between the lymph node scans, lymphangiograms, and exploratory laparotomy was poor, and the scan is not an adequate screening procedure. Averette et al.⁵ reported that of those patients with palpated negative para-aortic nodes, actually 12% to 13% contained metastatic disease. Pelvic and para-aortic node biopsies have increased in popularity because of these inadequacies. The incidence of pelvic node involvement in many gynecologic malignancies was deter-

Table V. Incidence of positive para-aortic nodes in endometrial adenocarcinoma as reported in the literature

Study	Patients	
	n	%
Creasman et al. ^{9*}	10/102	10
Delgado et al. ¹⁰	3/11	27
Piver et al. ^{11*}	6/41	15
Present study	7/90	7.8

*Stage I only.

Table VI. Incidence of positive para-aortic nodes according to uterine size

Study	Stage IA (%)	Stage IB (%)
Creasman et al. ⁹	3.8	11.7
Piver et al. ¹¹	3.8	33.3
Present study	4.5	9.1

mined several years ago. Because most gynecologic malignancies spread in an orderly and predictable manner from the pelvic to the para-aortic area, it is understandable that the para-aortic area would become the next level of investigation.

Several questions have arisen concerning para-aortic node biopsies. Today there seems to be little doubt that a para-aortic node biopsy is the most accurate method of assessing disease metastatic to this area. Many early studies were designed to investigate the correlation between the stage of the disease and the incidence of positive para-aortic nodes.⁶ There is factual data relating the incidence of positive para-aortic nodes and the stage of cervical carcinoma. Further information needs to be published relating the stages of endometrial adenocarcinoma and the incidence of positive para-aortic nodes. This study sheds some light on that question. However, some of the questions that remain concerning para-aortic node biopsies are (1) will therapy be modified by the results of a para-aortic node biopsy and (2) will the evidence gained from the para-aortic node biopsy improve patient survival? Our retrospective study addresses these two questions.

Para-aortic node biopsies were performed on 120 patients with cervical carcinoma. The studies of Nelson, Buchsbaum, Piver, and Averette have shown that in cervical carcinoma the incidence of para-aortic nodal involvement is directly related to the stage of the disease (Table IV).^{2, 6-8} Because of the potential and reported complications from para-aortic node irradiation, only those patients with positive para-aortic nodes should have para-aortic node irradiation. Therefore, the knowledge of the presence of positive para-aortic nodes will have an effect on therapy.

We performed para-aortic node biopsies on 90 patients with various stages of endometrial adenocarci-

Table VII. Incidence of positive para-aortic nodes according to histologic grade of endometrial adenocarcinoma

Study	Grade (%)		
	1	2	3
Creasman et al. ⁹	1.5	4.0	28.0
Piver et al. ¹¹	0.0	13.6	37.5
Present study	0.0	3.6	23.1

Table VIII. Incidence of positive para-aortic nodes in two studies of endometrial adenocarcinoma

Stage	Present study (%)	Creasman et al. ⁹ (%)
IAI	0.0	2.6
IAII	5.0	3.5
IAIII	25.0	8.3
IBI	0.0	0.0
IBII	0.0	4.8
IBIII	25.0	46.0

Table IX. Survival rates for cervical carcinoma patients with positive para-aortic nodes treatment

Study	% of survival		
	2 years	3 years	5 years
Buchsbaum ⁸	—	—	23.8
Hughes et al. ¹⁷	—	—	29.0
Ballon et al. ¹⁶	—	—	23.0
Berman et al. ¹⁵	—	25	—
Present study	18.1	—	9.1

noma. Seven of these 90 patients (7.8%) had positive para-aortic nodes. The incidence reported in the literature varies between 10% and 27%, as shown in Table V.⁹⁻¹¹ In 1976 Creasman et al. related the incidence of para-aortic nodal involvement to both uterine size and histologic grade. Our results and those of Piver et al. confirmed the original report of Creasman et al. that the histologic differentiation is of more prognostic value than the length of the uterus (Tables VI and VII). Table VIII lists the incidence of para-aortic node involvement for Stage I. As a result of these studies we are performing para-aortic node biopsies on all patients with endometrial adenocarcinomas, except Stage IAI disease. Considering the present information, a positive para-aortic node biopsy will affect the primary therapy of these unfortunate patients.

Whether the knowledge of para-aortic nodal involvement will improve the patient's survival remains the most important question. Some authors have stated that positive para-aortic nodes should not be irradiated because the volume of tissue involved does not allow the administration of a curative dose of irradiation. They

have predicted that irradiation therapy complications would be prohibitive, and early reports have supported this view.¹² Other authors have written that nodal disease indicates disseminated spread throughout the body and therefore is not curable.¹³ Our results would not support this view.

We must be certain that surgery and radiation therapy does not initiate more complications than the number of patients that survive. Most of the studies, and certainly ours, support the fact that surgical complications are minimal and minor. Only 2.4% of the 210 patients had surgical complications directly attributable to the biopsy, and these were all corrected immediately with no postoperative affect. Piver et al. early reported an unaccepted level of radiation therapy complications. Several authors have reported morbidities between 16% and 57% and mortalities between 5% and 15%.^{12, 14} These earlier studies demonstrated that 6000 rad resulted in severe radiation therapy complications. However, in our institution we have had no morbidity or mortality related to para-aortic irradiation.

Fourteen of our patients received between 4000 and 5000 rad; two patients received <4000 rad to the para-aortic area. This probably explains the reason for our low incidence of complications. Since our survival figures are consistent with others reported in the literature, 4000 to 5000 rad is probably an adequate treatment level for the small amounts of disease.

The question still remains, Does the survival rate exceed the combined surgical and irradiation therapy complication rate?

Twelve patients with carcinoma of the cervix had positive para-aortic nodes, and 11 were treated with radiation therapy. Three of those patients are alive without evidence of disease, and eight patients have succumbed to their disease, resulting in a survival rate of 25.0%. Table IX lists the comparative reported survival rates.¹⁵⁻¹⁸

Seven patients with endometrial adenocarcinoma had positive para-aortic nodes. Six of these patients were treated with irradiation therapy, and three are still alive. However, one patient with a positive para-aortic node was treated with chemotherapy and is still alive. This patient must be included in the survival rates, since a para-aortic node biopsy is not designed as a therapeutic procedure but rather as a diagnostic one. The patient would not have been treated had not the presence of positive para-aortic nodes been identified. Therefore, it is correct to include her in the survival group for endometrial adenocarcinoma. Therefore, four of seven patients with endometrial adenocarcinoma (57.1%) are still living. The actual 5-year survival rate is 28.6%. This compares with Potish et al.'s reported survival rate of 47.0%.¹⁹

Since this is a retrospective review without a control group of patients, we have not truly demonstrated in-

creased patient survival. Our results do indicate that appropriately treated patients with positive para-aortic nodes will survive.

In summary, our experience would indicate that (1) para-aortic node biopsy is a safe procedure when performed by adequately trained physicians, (2) radiation therapy can be administered safely to the para-aortic area provided that the total dose remains between 4000 to 5000 rad, (3) the disease in the para-aortic area does not indicate that the disease has always spread beyond that area, (4) with appropriate treatment there will be long-term survivors as a result of para-aortic node biopsy, and (5) this percentage of survivors is greater than the complication rate from radiation therapy and surgery.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. HOWARD W. JONES III, Nashville, Tennessee. I should like to address my remarks to the two questions that Dr. Blythe and his colleagues have posed in their paper: Will therapy be modified by the results of a para-aortic node biopsy? and, if so, will the modified therapy result in an improved survival?

Since the purpose of a "metastatic workup" in any patient with cancer is to determine the extent of disease so that a rational treatment plan can be devised, it seems self-evident that the answer to the first question should be "yes." If a positive aortic node biopsy would not influence therapy, there would be no purpose in doing the procedure. Many nonsurgical techniques for identifying metastatic cancer in the aortic lymph nodes have been tried over the years. Ultrasound, lymphangiograms, CT scans, and now nuclear magnetic resonance imaging have all been found inadequate to identify those patients with small foci of disease. Thus most gynecologic oncologists would agree that open biopsy is the most accurate way to evaluate the extent of disease, but because of the expense and morbidity involved, the selection of patients for this procedure must be addressed.

In his paper Blythe has presented a potpourri of patients with a variety of diagnoses. The criteria for patient selection were not stated, and it is difficult to sort them out and draw conclusions concerning the incidence of aortic lymph node metastases and the value of aortic lymph node biopsy in such dissimilar groups as squamous cell carcinoma of the vulva, adenocarcinoma of the endometrium, fascial fibromatosis, and unspecified ovarian cancer. Although he has demonstrated that the surgical morbidity is quite acceptable, the risk/benefit ratio varies considerably from one type of cancer to another. I would like to ask Dr. Blythe to comment on which patients with which cancers he recommends for an aortic lymph node biopsy?

Who should do the biopsy? At Vanderbilt our residents also participate in pelvic and aortic lymphadenectomies and biopsies, but I really do not consider them proficient enough to do aortic lymph node biopsies on their own. Many patients with ovarian cancer and most patients with endometrial cancer are operated on initially by a community-based gynecologist who has had little experience with aortic lymph node biopsies. Should we improve the surgical range of gynecologists in general or should we identify a group of patients who should be referred to gynecologic oncologists for operations?

Despite the fact that many investigators have reex-

plored the value of surgical staging in cervical and endometrial cancer for more than 10 years, we still do not have a clear answer to the question of whether it is beneficial or not. In an elegant paper Chism et al.¹ examined the theoretical potential gain in survival which could be anticipated in patients with cervical cancer if those women with aortic nodal disease could be identified and successfully treated. On the one hand, most patients with persistent or recurrent cervical cancer fail because of pelvic disease, and no amount of aortic radiation will cure that. On the other hand, a large number of patients with positive aortic nodes already have other distant metastatic disease on which aortic radiation will have no effect. For example, Buchsbaum² reported that 34.8% of his patients with positive aortic nodes also had positive scalene lymph node biopsies. In the theoretical analysis, even assuming 100% cure of aortic nodes, the overall net benefit was only 3% to 5% improvement in 5-year survival, and most of this was in patients with Stage IIB disease. Blythe and his colleagues have reported three of 12 patients with cervical cancer and aortic nodal disease are still and well at 16, 30, and 41 months. They actually only represent three of 120 women with cervical cancer who underwent operations. If these three patients survive 5 years, they would increase the overall survival only 2.5%—consistent with the calculations of Chism et al.

The outlook for patients with endometrial cancer may not be so poor, since pelvic disease is more easily controlled. I congratulate the authors on their fine work. They have achieved a very low surgical and radiation therapy complication rate, and their results give rise to cautious optimism. Continued long-term protocol studies of this nature will undoubtedly lead to a better understanding of gynecologic cancers, and better treatment techniques with improved survival rates will follow.

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DR. JOHN R. LURAIN, Chicago, Illinois. In recent years the para-aortic lymph nodes have come under closer scrutiny as sites of metastases in gynecologic malignancies. Gynecologic malignancies that were thought to be limited to the pelvis are now being found to have metastatic disease in the para-aortic lymph nodes in a significant number of cases. Para-aortic lymphadenectomy or biopsy of enlarged lymph nodes in the region of the aorta and vena cava, therefore, is finding increasing use in the evaluation of presumed localized gynecologic tumors. The finding of overt or subclinical metastases in the para-aortic lymph nodes in this group of patients may explain, in part, certain treatment failures with presently used treatment techniques. First, this knowledge partially explains the lack of significant

improvement in survival of locally advanced cervical cancer despite improved radiotherapy over the past 30 years. Second, para-aortic lymph node metastases have been implicated in the failure of pelvic radiotherapy to improve survival after operation in early endometrial adenocarcinoma and sarcoma. Third, unsuspected para-aortic lymph node metastases in ovarian cancer thought to be limited to the ovary may also partially explain why the 5-year survival rate is as low as 60% to 70% with early disease.

Dr. Blythe and his colleagues have presented their experience with para-aortic lymph node biopsy in 240 patients with gynecologic malignancies in order to assess the benefit of this procedure in terms of increased survival. Overall, only 22 (9.2%) of the 240 patients had positive para-aortic lymph nodes. Eighteen of these 22 patients received radiotherapy directed against these lymph node metastases, and eight (36.3%) of these patients were still alive at from 1 month to 67 months. From this information alone it is apparent that para-aortic lymph node biopsy had little impact on improving overall survival of the 240 patients in this report. If we analyze patients with cervical cancer and endometrial cancer separately, we may be able to get a clearer picture of the place of para-aortic lymph node biopsy in the assessment of these two malignancies.

Several series of pretherapy para-aortic lymph node biopsy for assessment of locally advanced carcinoma of the cervix have determined the incidence of para-aortic lymph node metastases to be 2% to 5% in Stage IB, 5% to 15% in Stage IIB, and 30% to 50% in Stage IIIB, or about 25% overall. Complications, especially intestinal injuries, from staging laparotomies and subsequent radiation therapy were significant in early reports. These complications have subsequently been reduced by use of an extraperitoneal approach and reduced doses of radiation therapy (usually about 4500 rad) to the para-aortic lymph nodes. The results of treatment directed against metastatic cervical cancer in para-aortic lymph nodes, however, remain poor. The 2-year survival without evidence of recurrence for women treated with para-aortic radiation for biopsy-proven para-aortic lymph node metastases is only about 20% to 25%.

In this study Blythe and associates operated on 120 patients with cervical cancer who would not otherwise have undergone a major surgical procedure in order to assess the status of the para-aortic nodes. Twelve patients (10%) were found to have para-aortic lymph node metastases. Of the 11 patients treated with radiotherapy directed to the para-aortic lymph nodes, only three have survived without evidence of disease. Thus, 120 patients were operated on to help three, or 2.5%, of all patients.

I believe that pretreatment staging laparotomy for biopsy of para-aortic lymph nodes in patients with cervical cancer is still an experimental procedure that should not be done routinely. The overall incidence of demonstrable para-aortic lymph node metastases by this technique is only approximately 25%, and we do not know how to cure a significant number of these

patients when we do find metastases in the aortic nodes. On the other hand, an alternative approach using lymphangiography and/or computerized tomography with thin-needle aspiration biopsy of suspected positive lymph nodes yields an unacceptably high false negative rate.

A case can be made for the use of routine irradiation of the para-aortic lymph nodes in locally advanced cervical cancer. The concept of prophylactic para-aortic irradiation is worthy of consideration because of (1) lack of acceptable and accurate diagnostic methods for detection of subclinical para-aortic disease, (2) the documented increase in morbidity with combined surgical procedure and irradiation, and (3) the fact that surgical staging is a sampling procedure at best, with microscopic disease often remaining undetected. Rotman et al.¹ attempted to determine the usefulness of such an approach by retrospectively evaluating 78 patients with Stages IIB, IIIB, and IVA cervical carcinoma who were treated with standard pelvic radiotherapy with or without para-aortic nodal irradiation (4500 rad). There was a significantly improved 20-year survival rate (79% versus 50%) in Stage IIB carcinoma of the cervix with the addition of para-aortic irradiation, without any concomitant increase in morbidity or mortality. There was no change in the 2-year survival rate in patients with Stage IIIB cervical cancer treated in a similar manner. This study suggests that the numerous failures to control disease in Stage IIB cervical cancer can, in part, be attributed to subclinical metastases to para-aortic lymph nodes, some of which can be eliminated by the radiation doses used in their study. In still higher stage patients, however, the tumor burden has probably advanced beyond control by local/regional treatment modalities because of such factors as bulk of pelvic and/or nodal disease, presence of extranodal metastases, and absence or failure of immunologic defenses.

Nori et al.² confirmed the increased success in treating cervical cancer patients with para-aortic metastases who had Stage I and II disease (eight of 17, or 47%) versus those with Stages III and IVA disease (none of 10, or 0%). Averette et al.³ and Rubin et al.⁴ also noted an increased survival in patients with Stage IB and IIA disease treated for para-aortic nodal metastases compared to patients with disease in more advanced stages. In Stages III and IVA disease there is no evidence yet that extended field irradiation will improve survival.

It is now recognized that certain women with early endometrial carcinoma are at risk for para-aortic lymph node metastases. A substantial risk for nodal metastases exists if there is deep myometrial invasion by cancer, if the tumor is grade 3 or of clear cell, papillary, or adenocarcinomatous histologic origin, or if there is tumor extension to the cervix and/or adnexa.⁵ Size of the uterus is less important in determining prognosis.

I agree with Blythe and his colleagues that initial treatment of all patients with Stage I endometrial cancer should consist of exploratory laparotomy, total abdominal hysterectomy, and bilateral salpingo-oophorectomy. Selected pelvic and aortic lymph node biopsies should be performed in patients with grade 2 and 3

tumors and in those with deep myometrial invasion or unexpected cervical or adnexal extension as determined by examination of the opened uterus at the time of operation. Postoperative radiotherapy can then be tailored to the surgical/pathologic findings. Patients with grade 1 and 2 tumors with superficial myometrial invasion can be treated with vaginal cesium postoperatively to eliminate the 3% to 10% incidence of local recurrence. Patients with grade 3 tumors, deep myometrial invasion, cervical extension, adnexal spread, or pelvic lymph node metastases should receive postoperative pelvic radiotherapy, usually 5000 rad.

The question that Blythe and associates are attempting to answer is, Can patients with endometrial cancer metastatic to the para-aortic lymph nodes be cured? The answer seems to be Yes, some of these patients can be cured. One half (three of six) of the patients in the present report treated with para-aortic radiotherapy for lymph node metastases are alive without evidence of disease. This result agrees with reports by Potish et al.⁶ and Komaki et al.⁷ of 5-year survivals of 43% and 60%, respectively, with use of postoperative para-aortic radiotherapy for aortic nodal metastases in endometrial cancer.

This study demonstrates that radiation therapy in doses of 4500 to 5000 rad can be delivered to the para-aortic area following biopsy of para-aortic lymph nodes containing metastatic cancer with minimal complications and with a 25% to 50% expectation of prolonged survival in cervical and endometrial cancer, respectively. This report raises several questions that still need to be answered. Which patients with aortic nodal metastases can be cured? Does presence of microscopic rather than clinical nodal metastases make a difference? Should para-aortic lymph node biopsies continue to be performed for evaluation of locally advanced cervical cancer in patients who would otherwise not be undergoing a major operative procedure? Does irradiation of the para-aortic lymph nodes without biopsy-proven disease in cervical cancer have a greater morbidity than pretherapy aortic lymph node biopsy followed by radiotherapy in only patients with biopsy-proven disease? Could the survival rate in Stages IB and II cervical cancer be improved with prophylactic para-aortic irradiation rather than reliance on imperfect biopsy and radiologic techniques to target patients for para-aortic radiotherapy? Which patients might benefit from adjuvant chemotherapy in addition to extended field irradiation? Have Blythe and colleagues used neoadjuvant chemotherapy such as cisplatin and 5-fluorouracil in cervical cancer or melphalan, 5-fluorouracil, and progestins or tamoxifen in endometrial cancer in addition to pelvic and aortic irradiation in patients with disease metastatic to the para-aortic lymph nodes? I would appreciate Dr. Blythe's comments on these questions.

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DR. EDUARD EICHNER, Cleveland, Ohio. In the early 50s I went to Boston to learn to perform radical surgery with Dr. Meigs; at that time I took along my lymphatic studies, which so interested him that he later published them in *Progress in Gynecology* (volume III) in the mid-50s. This resulted in a change in his technique, which became known as the Meigs' hysterectomy, in which he removed the parasacral nodes up to the bifurcation of the aorta and picked up the nodes at the rim there. He told me about 10 years later that once he learned the surgical technique and had solved that problem, his salvage rate and his success rate improved.

DR. VINAY MALVIYA, Detroit, Michigan. What was the extent of periaortic lymph node dissection carried out during the staging procedure?

How many patients with endometrial cancer with positive periaortic lymph nodes had other sites of metastatic disease?

What was the treatment offered to patients with ovarian cancer with positive periaortic nodes? Was radiation therapy employed in addition to chemotherapy in these patients?

DR. BLYTHE (Closing). Let me begin with Dr. Jones' comments. He asked if I would comment on which patients with cancer I recommend having a periaortic node biopsy. I do not think I am in a position yet to make that recommendation. All of the patients in our institution are treated according to protocol, and the protocol for all patients includes a staging laparotomy. At the time of the staging laparotomy if there are no contraindications such as obesity, a periaortic node biopsy is performed.

Who should do the biopsy? I think this is limited to the specific setting. In our hospital there are two full-time gynecologic oncologists plus 50 private physicians in the department of obstetrics and gynecology, and we feel that gynecologic oncologists should perform these biopsies. Your situation may be different.

Should we improve the surgical ability of gynecologists in general or should we identify a group of patients who should be referred? We probably should identify the appropriate patients and maintain the surgical procedure for these particular patients.

I do not think we can retrain everybody, and I do not believe residents should be encouraged to think they can do this particular procedure, although our third-year residents do it. We do that for a variety of reasons. It gives us an opportunity to discuss the anatomy with them. It gives them an opportunity to see the anatomy. It gives us the opportunity to discuss the importance of metastatic disease and how it spreads. It gives them the opportunity to sharpen their surgical skills with procedures that they otherwise would not have the opportunity to perform. It gives them a sense of security. We do not, in any way, leave them with the idea that they are qualified to do the biopsies, for two reasons: (1) they do not get themselves out of trouble, so they have no experience in doing that; (2) they do not perform enough biopsies to gain the expertise that is necessary.

Have we identified any characteristics associated with a favorable prognosis in cervical cancer patients? No. There are other studies. I believe Dr. Buchsbaum has done one, as have others, that show that staging laparotomies not only show evidence of metastatic disease in the periaortic areas but also in the liver, the bowel, the omentum, not only for cervical carcinomas but for others, so I have some problem with doing away with staging laparotomy.

I am also concerned about prophylactic periaortic irradiation for a couple of reasons. I have a problem with prophylactic almost-anything. The literature is re-

plete with deaths caused by periaortic irradiation; therefore, I think it is dangerous to irradiate this area without histologic proof that disease is present.

Dr. Eichner, that was an interesting comment. I think periaortic node biopsy is probably about where pelvic lymph node biopsy was a number of years ago. We are finding out what is there. We have to find that out first. Then we have to decide how we are going to treat this. What treatment modality is successful? Finding these two things, we can then begin to pick out the subsets of patients who will be helped and those who will not. We did that with pelvic lymph nodes. Now most of you will probably give some form of pelvic irradiation to patients with Stage 2 endometrial carcinoma and do not bother to biopsy the pelvic lymph nodes because the answer has already been provided for you. I think we are about at that same stage, working through that process with periaortic node biopsies.

In answer to Dr. Malviya's question, our biopsies go from the mid-common iliacs to the origin of the inferior mesenteric artery.

Was there evidence of other metastatic disease in patients? No, although we did do some supraclavicular node biopsies on these patients. Our staging laparotomies really have not indicated evidence of other areas of metastasis. We treat positive periaortic nodes in carcinoma of the ovary with chemotherapy, our standard PAC-1 regimen, but we do add periaortic irradiation therapy.

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Pelvic lymphadenectomy in operative treatment of ovarian cancer

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From the end of 1979 to September, 1985, radical pelvic lymphadenectomy was performed at the Graz Clinic in 123 cases of Stages IA to IV ovarian cancer following maximum debulking procedure. In 97 patients lymphadenectomy was done primarily. In 26 it was performed during a follow-up operation to chemotherapy. The frequency of pelvic node involvement was 61.8% in the total material and 78.0% in 82 cases of Stage III disease only; 75.0% positive nodes were found in Stage III after chemotherapy. Aortic nodes were positive in 41.4%, but only when pelvic nodes were also positive. The 5-year actuarial survival rate for Stage III disease was 53.0% after pelvic lymphadenectomy compared with 13.0% without. In cases with negative nodes the survival rate was 74.7%; with positive nodes the survival rate was 45.9%. (AM J OBSTET GYNECOL 1986;155:315-9.)

Key words: Ovarian cancer, pelvic node involvement, lymphadenectomy, survival rates

The results of the treatment of ovarian cancer have been the poorest in gynecologic oncology, when cancer of the Fallopian tubes is disregarded. The favorable effects of chemotherapy seemed to offer hope for resolving this problem, but expectations were not realized because the achieved prolongation of life was not synonymous with true survival. However, the introduction of chemotherapy presented a challenge to increase surgical efforts because a close relationship between chemotherapy and residual tumor mass became apparent. Debulking was a logical consequence of this knowledge. The routine operation for ovarian cancer that consisted of total abdominal hysterectomy with adnexectomy and omentectomy was continuously increased, finally reaching the extent of a major operation. Within the continuous development of the treatment protocols at the Graz Clinic, the management changed from total abdominal hysterectomy and bilateral salpingo-oophorectomy with occasional omentectomy and a not-too-extensive debulking procedure to a drastic debulking with the compulsory resection of involved portions of the bowel, bladder, and parietal peritoneum. Simultaneously the chemotherapeutic regimen changed from the melphalan and Adriamycin-cyclophosphamide (AC) regimen to the PAC regimen, containing cisplatin, Adriamycin, and cyclophosphamide.

Table I. Age distribution and pelvic lymph node involvement in 123 patients with ovarian cancer

Age (yr)	No. of patients	No. with involvement
21-30	2	1
31-40	6	2
41-50	36	25
51-60	46	32
61-70	28	13
71-80	5	3
Total	123	76

To date, routine total pelvic lymphadenectomy has not been performed for ovarian cancer. Available reports are based on a sampling of lymph nodes only. Therefore different figures concerning the frequency of pelvic lymph node involvement have been obtained, depending on the intensity of exploration. In 1978 Creasman et al.¹ described four cases with positive nodes. Averette et al.,² on the other hand, found pelvic node involvement in 27.1% and aortic node involvement equally in 27.1%. Chen and Lee³ reported 14.8% positive pelvic nodes and 37.7% positive aortic nodes in their cases.

The anatomy of ovarian lymph drainage is complex. It has been stated that the drainage trunks leaving the subovarian plexus take a cephalad course toward the aortic nodes via the infundibulopelvic ligament "almost exclusively."⁴ Little attention has been paid to a lymphatic channel that courses from the hilus of the ovary within the folds of the broad ligaments to the interiliac nodes, described by French authors.^{5,6} These nodes are interconnected by a great variety of anastomoses with external, medial, and deep common iliac nodes, as well

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Table II. Frequency of bowel resection and size of residual tumor within debulking procedure, 1979 to September, 1985

Stage*	No. of patients	Bowel resection		Residual tumor					
				None		<2 cm		>2 cm	
		n	%	n	%	n	%	n	%
III	70	22	31.4	29	41.4	14	20.0	27	38.6
IV	8	4		2		1		5	
Total	78	26	33.3	31	39.7	15	19.2	32	41.0

*International Federation of Gynecology and Obstetrics.

Table III. Pelvic node involvement in ovarian cancer, 1979 to September, 1985

Stage*	No. of patients	No. with involvement	%
IA	8	3	37.5
IB	3	3	100.0
IC	12	1	8.3
IIA	1	—	—
IIB	7	—	—
III	82	64	78.0
IV	10	5	50.0
Total	123	76	61.8

*International Federation of Gynecology and Obstetrics.

Table IV. Pelvic node involvement in primary lymphadenectomy, 1979 to September, 1985

Stage*	No. of patients	No. with involvement	%
IA	5	—	—
IC	8	—	—
IIA	1	—	—
IIB	5	—	—
III	70	55	78.6
IV	8	4	50.0
Total	97	59	60.8

*International Federation of Gynecology and Obstetrics.

as with the aortic nodes. Because of this interconnection, the latter nodes may "become secondary nodes draining the various pelvic organs."¹

Consequently the role that a route of spread may have in the lymphatic dissemination of ovarian cancer is difficult to determine. In 1954 Eichner and Bove⁷ proposed pelvic lymphadenectomy in ovarian cancer based on in vivo studies of the lymphatic drainage of the human ovary. In 1966 Bergmann⁸ showed in autopsy that patients who died of ovarian cancer had both pelvic and aortic node involvement with practically the same high frequency, 80%. Clinical studies are needed to clarify in which sequence lymphatic dissemination usually occurs.

The purpose of sampling lymph nodes is to obtain a better knowledge of cancer spread and therefore a better understanding of the prognosis of a case. This is the same purpose for sampling lymph nodes in cases of advanced cervical cancer.⁹ Radiation protocols are based on the knowledge thus gained. Within the radical operation for cervical cancer, total pelvic lymphadenectomy, which may be extended to the aortic region, is part of our effort to eliminate every possible cancer location. Should it appear therefore that lymph node involvement, especially involvement of pelvic nodes, is very frequent in ovarian cancer, a complete lymphadenectomy would result.

Material and methods

From the end of 1979 to September, 1985, systematic pelvic lymphadenectomy was performed at the Graz Clinic in 123 cases of Stages IA to IV ovarian cancer. Table I shows the age distribution as well as the pelvic lymph node status in the individual age groups. With regard to lymphadenectomy, two groups are distinguished. In 97 patients lymphadenectomy was part of the primary operative treatment. We will call this "primary lymphadenectomy." In 26 patients whose primary operation was done within 1 year before the introduction of routine lymphadenectomy or who were treated primarily by chemotherapy only, lymphadenectomy was performed during second-look laparotomy. We will call this "secondary lymphadenectomy." In each case the most extensive debulking possible was done as well as total hysterectomy with adnexectomy and omentectomy.

Table II shows the frequency of bowel resection in 78 cases of Stages III and IV disease in which primary lymphadenectomy was performed. It also demonstrates the extent that it was possible to clear the abdomen completely of macroscopically visible cancer or in how many cases remnants were <2 cm or >2 cm in diameter. All but one were treated postoperatively with the PAC regimen.

Cases of Stage III disease were divided into the fol-

Table V. Incidence of pelvic lymph node involvement in proposed subgroups of Stage III, 1979 to September, 1985

Stage*	No. of cases	No. with involvement	%
IIIA	16	13	81.3
IIIB	9	5	55.6
IIIC	45	37	82.2

*International Federation of Gynecology and Obstetrics.

lowing subgroups according to a proposal by Ulfelder (personal communication): Stage IIIA, microscopic spread to upper abdomen; Stage IIIB, implants not larger than 2 cm; and Stage IIIC, all others.

Lymphadenectomy was always performed as extensively as possible. We started at the femoral ring and followed the external and common iliac vessels. The node-containing tissue was first dissected on the ventral side of the vessels up to the aortal bifurcation. The subaortic and gluteal nodes were then checked or removed. Next the obturator fossa was cleared after localization of the obturator nerve by bluntly separating the lymph node-containing tissue with the medial external iliac and obturator nodes from the posterior wall of the iliac external vein and from the fascia of the obturator muscle. The obturator vessels were resected completely so that the remaining tissue could be removed from the levator muscle. Then the iliac vessels were loosened from the pelvic wall to complete lymphadenectomy in the upper part of the obturator fossa and to remove the deep external iliac and deep lateral and medial common iliac nodes that were partly covered by the veins. The iliac external vein and artery were separated from one another and carefully cleared.

Aortic lymphadenectomy was not performed in every case and not as thoroughly as pelvic lymphadenectomy. The node-containing tissue was removed from the frontal and lateral sides of the vessels whenever possible. This was usually done up to the level of the mesenteric artery. In the more cephalad parts the procedure was rather a sampling of palpable enlarged nodes than a curative operation. Recently sampling of aortal nodes has been replaced as much as possible with total removal up to the renal vessels. Such cases are not herein presented.

In 97 cases the average time of operation was 4.33 hours, with a range from 1.40 to 8.00 hours. The survival rates (or survival probabilities) were calculated by means of the actuarial life table method.¹⁰ For comparing various survival curves the Logrank test¹¹ was applied. The evaluations were effected with the HP-IL system of the Institute of Mathematics of the University of Klagenfurt.

Table VI. Histologic types of ovarian cancer and frequency of pelvic lymph node involvement with primary operation

	No. of patients	No. with involvement	%
Serous	60	40	66.7
Mucinous	5	1	20.0
Endometrial	14	6	42.9
Mesonephric	9	5	55.6
Undifferentiated	8	7	87.5
Embryonal	1	—	—
Total	97	59	60.8

The advantage of establishing the rate by this method as opposed to using the classical reduced-sample rates lies in the ability to use all given data. For example, by use of this method, patients observed <3 years, for example, 1 year, may be included in the 3-year rate. This also allows an improvement in the intrinsic value of thus-calculated rates, expressed in a reduction in confidence intervals. This reduction is pointed out by Cutler and Ederer.¹⁰ In the present investigation the majority of patients (in the group with radical operations with lymphadenectomy, that is, 72.7%) were within the 0 to 3 year range, so that the 4- and 5-year rates are statistically rather poorly supported. Slight alterations in the absolute survival numbers may thus cause major fluctuations in the respective rates. The Logrank test is nevertheless applicable.

Results

Positive pelvic nodes were found from Stage IA cancers in 61.8% of cases. In Stage I, however, these were found only in secondary lymphadenectomy. Statistically relevant figures are available for Stages III and IV, which cover the greater part of our material (Table III).

In contrast to the foregoing, in 29 cases of Stages III and IV disease in which the para-aortic nodes were removed or sampled, the frequency of nodal involvement was 41.4%. In contrast to the findings of Chen and Lee,³ however, positive para-aortic nodes were seen only in cases with pelvic nodal involvement as well.

If primary lymphadenectomy is being considered alone, positive pelvic nodes were detected in Stages III and IV only, with 78.6% frequency in Stage III (Table IV). Table V shows that the percentage of positive nodes was highest in Stage IIIC but almost equally high in Stage IIIA. The lower frequency in Stage IIIB may be due to a smaller number of cases. Table VI shows the frequency of pelvic node involvement with regard to tumor type.

Following the introduction of systematic pelvic lymphadenectomy, 26 patients with Stages IA to IV

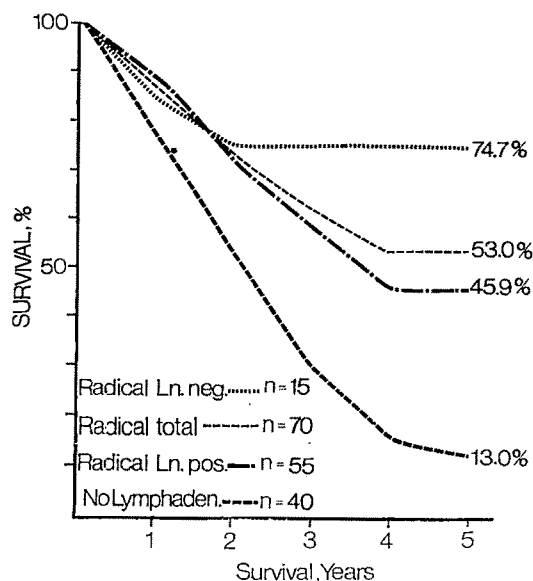


Fig. 1. Actuarial survival rates in Stage III ovarian cancer after total abdominal hysterectomy and bilateral salpingo-oophorectomy with omentectomy and debulking compared with debulking and radical pelvic lymphadenectomy.

Table VII. Pelvic node involvement in secondary lymphadenectomy, 1979 to September, 1985

Stage*	No. of patients	No. with involvement	%
IA	3	3	100.0
IB	3	3	100.0
IC	4	1	25.0
IIB	2	—	—
III	12	9	75.0
IV	2	1	50.0
Total	26	17	65.4

*International Federation of Gynecology and Obstetrics.

disease were admitted for second-look laparotomy after chemotherapy. Since no primary lymphadenectomy was performed in these cases, a secondary lymphadenectomy was done. Eight patients in Stage I underwent chemotherapy with melphalan while in two Stage I cases and one Stage III case the AC regimen was applied. All others were treated according to the PAC regimen. In spite of this, positive pelvic lymph nodes were revealed with a frequency that is not statistically different from that found before chemotherapy (Table VII).

During the period from 1976 to September, 1985, total abdominal hysterectomy and bilateral salpingo-oophorectomy, together with omentectomy and tumor debulking, was performed in 40 Stage III cases. This was followed by a complete cytotoxic treatment with use of the AC regimen in 13 cases and the PAC regimen in 27. The actuarial survival rate after 5 years was 13.0% in these cases (Fig. 1).

Following the routine pelvic lymphadenectomy, the

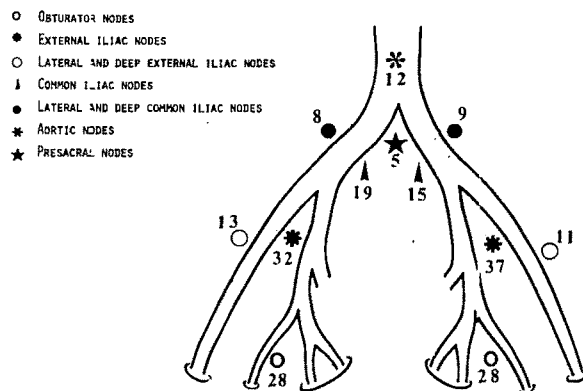


Fig. 2. Distribution of positive pelvic nodes in 123 cases of ovarian cancer.

treatment results showed a striking improvement. In Stage III the actuarial survival rates increased dramatically when compared with procedures before the introduction of lymphadenectomy. The improvement was particularly impressive in cases with negative nodes. Even in cases of positive nodes it was surprisingly high (Fig. 1). Table VIII indicates how the prognosis is dependent on age.

Comment

The crucial question is whether lymphadenectomy will decisively influence the cure rate in ovarian cancer.

The value of radical lymphadenectomy is substantiated by the survival rates. On comparing the Stage III cases with and without lymphadenectomy, differences appear which are more far-reaching than expected. Undoubtedly the compatibility of the two groups is relative because the treatment of ovarian cancer is continuously evolving. Both the operative treatment and chemotherapy have become more aggressive.

However, there are 17 less radically treated cases dating to the years 1976 to 1979 which were based on a more suitable selection as to operability. Thus this group as a whole should have even better results than the more recent cases with lymphadenectomy because, from 1976 to 1979, 34 of 51 Stage III cases (66.6%) were merely biopsied, whereas from the end of 1979 to September, 1985, such a restricted procedure was done in only 17 of 111 (15.3%) (Table IX).

Therefore probably none of these factors affected survival rates to the extent of the series compared. Moreover, the literature makes no indication of such an effect in the latest treatment protocols in spite of increased surgical efforts and the use of more aggressive cytostatic drugs.

On the other hand, it seems logical that leaving positive lymph nodes will influence results unfavorably, unless we may rely on the postoperative chemotherapy to influence lymph node metastases. According to our results, this is not the case. In cases with secondary

Table VIII. Age distribution and actuarial survival rates in Stage III ovarian cancer

	Age (yr)				
	31-40	41-50	51-60	61-70	71-80
No. of patients	3	21	28	15	3
Years of survival (%)					
1	100	83.8	100	68.0	66.7
2	100	69.2	78.4	54.4	66.7
3	100	37.3	78.4	54.4	66.7
4	100	37.3	58.8	54.4	66.7

Table IX. Operative treatment, International Federation of Gynecology and Obstetrics Stage III

	TAH + BSO (omentectomy)	Radical treatment with lymphadenectomy	Biopsy only	%
1976-1979	17	—	34	66.6
1979-Sept. 1985	24	70	17	15.3

TAH, Total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy.

lymphadenectomy, that is, after operation and chemotherapy (Table VII), we found no statistically significant difference in the frequency of lymph node involvement in Stages III and IV as compared with cases in which primary lymphadenectomy was done (Table IV).

The critical question is from which stage onward should pelvic lymphadenectomy be performed. In Stages IA and IB it is done only with exceptionally large tumors. In Stages I and II, however, no positive nodes were found in doing primary lymphadenectomy to this point (Table IV). Positive cases in secondary lymphadenectomy might be accredited to an inaccurate staging in older cases (Table VII). Although it may thus appear that lymphadenectomy should be performed in Stages III and IV only, we still continue performing this procedure in cases of Stage I and II.

The question cannot yet be answered as to the way in which recurrences come about in cases in which all intraperitoneal implants have been eradicated and in which cancer remained in the lymph nodes only. From experience, recurrences usually occur intraperitoneally and rarely retroperitoneally. On the other hand, it is unlikely that cancer within the nodes will remain arrested as if the nodes would assume the role of blocking further cancerous spread to the organism.

According to our present knowledge, the accessible pelvic nodes are involved primarily in gynecologic cancers and ovarian cancer. In the latter the external iliac and obturator nodes are more likely to be involved than the common iliac nodes (Fig. 2). This may also indicate the secondary role of cephalad nodal groups, including the aortic nodes. Major importance may be attributed to the interiliac nodes on account of their intercommunication with the iliac as well as the aortic groups.⁴ Special attention should be paid to the deep external iliac and deep lateral as well as medial common iliac

nodes, which are sometimes disregarded yet are frequently metastatically involved (Fig. 2).

Positive aortal nodes were not found in cases with negative pelvic nodes. It appears therefore that aortal nodes would be involved only followig involvement of pelvic nodes. This may be explained simply because the lymphatic stream is directed cephalad, particularly in consecutive nodal groups, such as the iliac and aortal nodes.

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Radioimmunoassay of free β -subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy

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A new radioimmunoassay system was established with a monoclonal antibody (1E5) that distinguishes the free β -subunit of human chorionic gonadotropin in the presence of intact human chorionic gonadotropin, showing only 0.23% cross-reactivity with the intact human chorionic gonadotropin molecule and virtually no cross-reactivity with other glycoprotein hormones or their β -subunits. Serum samples, taken at initial diagnosis from nine patients with hydatidiform mole and spontaneous remission and 12 patients with subsequent progression to persistent trophoblastic disease, were assayed for free and total levels of the β -subunit of human chorionic gonadotropin. The assay results were expressed as a ratio of nanograms of free β -subunit per 1000 mIU of total β -subunit. Eight of nine patients with mole and spontaneous remission had a ratio value <4 whereas 10 of 12 patients with subsequent persistent disease had a ratio value >4 . Statistical analysis with χ^2 showed a highly significant correlation of high ratios with eventual progressive disease ($p = 0.0009$). This study suggests that excessive production of the free β -subunit of human chorionic gonadotropin may identify patients with a high likelihood of developing persistent trophoblastic disease. (AM J OBSTET GYNECOL 1986;155:320-4.)

Key words: Monoclonal antibodies; free β -hCG, gestational trophoblastic disease, persistent

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two dissimilar non-covalently linked subunits of α - and β -chains. The α -chain is nearly identical to and immunologically indistinguishable from the α -subunit of some other pituitary glycoprotein hormones, including luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone. The β -subunits of these hormones differ in their amino acid sequences and in this way confer unique immunologic and biologic activity to each hormone.¹ The β -subunit of hCG (β -hCG) contains an extended COOH terminal of 30 amino acid residues not present in luteinizing hormone or other hormones. Conventional β -hCG assay systems use antibodies that are raised against the structural differences conferred on β -hCG by these unique amino acid sequences.² Today, these conventional β -hCG assays, while specific for β -hCG, do not discriminate the free β -subunit of hCG from intact hCG molecules. We have

developed a monoclonal antibody (1E5) that distinguishes the free β -hCG in the presence of hCG and shows only 0.23% cross-reactivity with the intact hCG molecule and virtually no cross-reactivity with other glycoprotein hormones or their β -subunits. A radioimmunoassay system has been established with this antibody.³

At the present time, serial determination of serum β -hCG concentrations, including measurement of both intact and free β -hCG, is the standard follow-up procedure after the initial diagnosis and suction curettage of patients with hydatidiform mole.⁴⁻⁷ Approximately 85% of these patients undergo spontaneous remission as evidenced by a gradual fall in the serum β -hCG level while 15% of patients develop persistent trophoblastic disease with a progressive rise in the serial levels. In either case, the level of β -hCG is a function of the half life of serum hCG and the rate of production of hCG by the trophoblastic tissue remaining in the uterus.

The hCG polypeptide and variable quantities of its subunits have been found in tumors containing trophoblastic tissue. Some tumors secrete only one form of the hCG subunits and others secrete a combination of the hormone and its subunits. Secretion of only the α -subunit of hCG in the absence of either β -subunit or the intact hCG molecule has been reported in a tumor of the stomach.⁸ Metastatic testicular choriocarcinoma also demonstrates differential production of hCG sub-

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Table I. Patients with hydatidiform mole who underwent spontaneous remission

Patient No.	Free β -hCG (ng/ml)	Total β -hCG (mIU/ml)	ng Free β -hCG/1000 mIU hCG
1	10.5	5,100	2.1
2	84.2	52,290	1.6
3	143.3	462,100	0.3
4	139.9	165,560	0.9
5	14.1	8,716	1.6
6	8.9	3,927	2.3
7	11.2	1,902	5.9
8	27.5	23,000	1.2
9	7.4	9,600	0.7
Mean $\pm$ SD	49.6 $\pm$ 57.3	81,355 $\pm$ 152,009	1.8 $\pm$ 1.7

Table II. Patients with hydatidiform mole who developed persistent trophoblastic disease

Patient No.	Free β -hCG (ng/ml)	Total β -hCG (mIU/ml)	ng Free β -hCG/1000 mIU hCG
1	4550	401,900	11.3
2	41.8	5,927	7.1
3	47.7	14,670	3.3
4	86.8	12,674	6.8
5	501	480,000	1.0
6	27.2	3,236	8.4
7	44.6	8,295	5.4
8	41.3	6,102	6.8
9	19.7	4,308	4.6
10	107.5	20,640	5.2
11	24.2	3,049	7.9
12	46.1	3,865	13.7
Mean $\pm$ SD	461 $\pm$ 1294	80,388 $\pm$ 169,322	6.8 $\pm$ 3.4

units with a high ratio of free β -hCG to total hCG molecule compared with that of normal pregnant subjects.⁹ The intact hCG secreted by these neoplastic tissues has not been shown to be different from that found secreted by the normal placenta.

The known clinicopathologic features of hydatidiform mole coupled with the knowledge that unbalanced synthesis of hCG subunits can occur in tumors with trophoblastic elements suggested that the quantitative measurement of hCG subunits might be useful in the laboratory evaluation of patients with molar pregnancy. This retrospective study was designed to provide preliminary data on whether measurement of the free β -hCG fraction in sera taken at the time of molar evacuation is useful in predicting which patients will develop persistent trophoblastic disease.

Material and methods

Serum samples obtained at initial diagnosis of molar pregnancy from 21 patients were selected from the serum bank of the Southern Regional Trophoblastic Disease Center. The selection was based on the patient having adequate serial follow-up to clearly delineate the eventual outcome in regard to spontaneous regression (nine patients) or persistent trophoblastic disease (12 patients). The serum specimens had been stored at

-20° C for periods ranging from 2 to 5 years. Samples were assayed for free β -hCG and total β -hCG individually without knowledge of clinical outcome of the patients or assay results of either component; that is, free β -hCG was assayed without knowledge of total β -hCG determination.

The free β -hCG assay was carried out by means of the double-antibody technique of Midgley.¹⁰ All reagents were diluted in phosphate-buffered saline containing 0.1% gelatin. The monoclonal antibody to free β -hCG produced in mice ascites was used at a dilution previously found to bind 30% of the added iodinated free β -hCG. The total assay volume was kept constant at 1.0 ml and included the following: 0.1 ml of unlabeled free β -hCG (standard) or samples; 0.1 ml of iodine 125-labeled free β -hCG (50,000 cpm); 0.2 ml of 1E5 monoclonal antibody diluted as mentioned above; 0.1 ml of normal mouse serum diluted at 1:100 in gel-phosphate-buffered saline; 0.1 ml of normal human serum. Gel-phosphate-buffered saline was used to bring the total volume to 1.0 ml. For standards and nonspecific binding controls, normal human serum was diluted to match the dilution of samples used in the assay. This reaction mixture was incubated for a minimum of 4 hours at room temperature. Then 1 ml of the second antibody, sheep antimouse γ -globulin, was

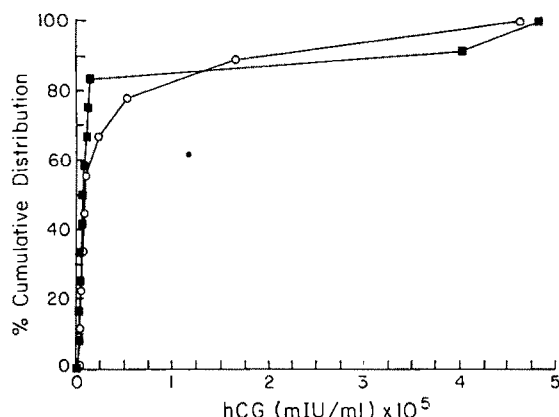


Fig. 1. Cumulative distribution of total hCG concentration in serum of patients with spontaneous remission (○) and those with subsequent persistent trophoblastic disease (◼).

added at a dilution of 1:100 in 2.5% polyethylene glycol and the incubation was continued for a minimum of 30 minutes at room temperature. Assay tubes were centrifuged at $7000 \times g$ at $4^\circ C$ for 20 minutes, decanted, and blotted, then counted in a Micromedics gamma counter (Micromedics Systems, Inc., Horsham, Pennsylvania). Data plots and calculations were performed on an MACC Micromedics computer interfaced with the gamma counter, with the use of a logit plot as previously described.³

Total β -hCG was assayed with a commercially available immunoradiometric system for hCG (Serono Diagnostics, Randolph, Massachusetts). Patient samples, standards, and controls, respectively, were reacted with a mixture of three monoclonal antibodies to hCG. The antibodies reacted with both free β -subunit and the intact hCG molecule. Two of the antibodies were labeled with ^{125}I and the third linked to fluorescein. Incubation was 15 minutes at $37^\circ C$. After incubation, antfluorescein coupled to a magnetic solid phase was added in large excess to bind to the hCG-monoclonal antibodies complex, which was precipitated in a magnetic field. The sensitivity of the assay is 5 mIU/ml of hCG. Calibration was against the first international reference preparation 75/537. Cross-reaction with luteinizing hormone and follicle-stimulating hormone was 0.55% and 0.68%, respectively. Precision of the assay, as expressed by interassay and intra-assay coefficients of variation, was <10% each.

The results on each sample were analyzed as the amount of free β -hCG (nanograms per milliliter), the amount of total β -hCG (milli-international units per milliliter), and a ratio of nanograms of free β -hCG per 1000 mIU of hCG. The sensitivity (or true positive rate) of the test was defined as the proportion of patients who had progressive disease and who were correctly identified by the test. The specificity (or true negative

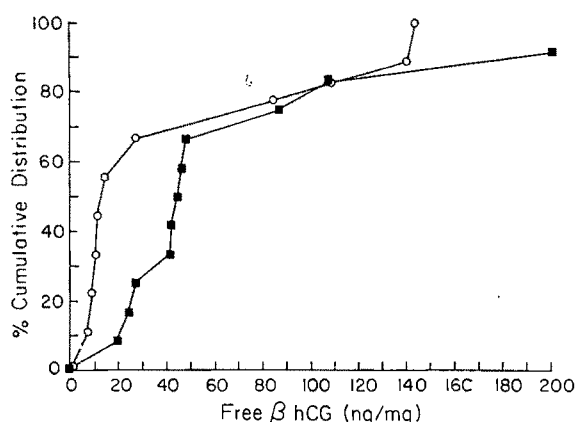


Fig. 2. Cumulative distribution of free β -hCG concentration in serum of patients with spontaneous remission (○) and those with subsequent persistent trophoblastic disease (◼).

rate) of the test was defined as the proportion of patients who had spontaneous regression and were correctly identified by the test. The significance of the sensitivity and specificity of the test was evaluated with the use of χ^2 and Wilcoxon two-sample test statistical analysis.¹¹

Results

The serum β -hCG values from patients who eventually developed spontaneous remission or persistent trophoblastic disease are listed in Tables I and II, respectively. Samples of serum had been stored for varying periods ranging from 2 to 5 years without significant differences in storage time between the two groups of patients. A wide range of free and total β -hCG values were noted in the two groups of patients. Both groups had similar mean total β -hCG values. The group of patients with progression had a higher mean value for free β -hCG, but it was not statistically significant ($p = 0.10$). The higher mean ratios of free β -hCG per 1000 mIU of hCG in the group with progression were highly significant when analyzed by the Wilcoxon two-sample test ($p = 0.002$).

When the cumulative distributions of patients with spontaneous remission (○) were compared with those of patients with persistent disease (◼) with the use of the values for total β -hCG (Fig. 1), free β -hCG (Fig. 2), and the ratio of nanograms of free β -subunit 1000 mIU hCG (Fig. 3), only the ratio values produced significant discrimination by the Wilcoxon two-sample test ($p = 0.002$).

Table III examines the sensitivity and specificity of identification of patients who will develop persistent trophoblastic disease with the use of cutoff ratio values of either 3.0 or 4.0 ng of free β -hCG per 1000 mIU of total β -hCG as selected from the cumulative distribution curves in Fig. 3. With a cutoff of 3.0, the true

Table III. Prognostic specificity of the assay of free β -hCG/1000 mIU of hCG with the use of cutoff values of 4.0 or 3.0

	Free β -hCG/1000 mIU hCG		Total
	<4.0 (<3.0)	>4.0 (>3.0)	
Spontaneous remission	8 (8)	1 (1)	9
Persistent trophoblastic disease	2 (1)	10 (11)	12
Total	10 (9)	11 (12)	21

$p = 0.0009$ (0.0002).

positive rate was 11/12 (92%) and the true negative rate was 8/9 (89%) with a p value of 0.0002 by the χ^2 test. With a cutoff of 4.0, the true positive rate was 10/12 (83.3%) and true negative rate was 8/9 (89%) with a p value of 0.0009.

Comment

Hydatidiform mole occurs in approximately 1 in 1200 pregnancies in the United States.⁴ Approximately 80% to 85% of women with hydatidiform mole will undergo spontaneous remission and 15% to 20% of women will develop persistent trophoblastic disease. The diagnosis and treatment of patients with persistent trophoblastic disease are based on the serial serum β -hCG titers. Patients with nonmetastatic and low-risk metastatic persistent trophoblastic disease are ultimately cured with single-agent or multiagent chemotherapy.^{12, 13} High-risk metastatic trophoblastic disease develops in approximately 4% of patients with hydatidiform mole. Survival for high-risk patients is about 60%. High-risk patients are treated with multiagent chemotherapy with resultant significant morbidity.¹³

Goldstein et al.⁵ treated 247 patients with prophylactic chemotherapy at the time of evacuation of the hydatidiform mole and had a 4% persistent disease rate while 858 patients who did not receive prophylactic chemotherapy had an 18.6% persistent disease rate. Those patients who developed persistent disease after receiving prophylactic chemotherapy all achieved complete remission with only one subsequent course of chemotherapy and none developed metastatic disease. It is apparent therefore that prophylactic chemotherapy can: (1) reduce the incidence of persistent trophoblastic disease, (2) decrease or eliminate metastatic trophoblastic disease, and (3) decrease the number of chemotherapy cycles needed if persistent disease does occur. Despite these demonstrated advantages, prophylactic chemotherapy has received little enthusiasm since 80% to 85% of patients undergo spontaneous remission and therefore would be treated unnecessarily.

For this reason, several investigators have attempted to identify a subset of patients with molar pregnancy

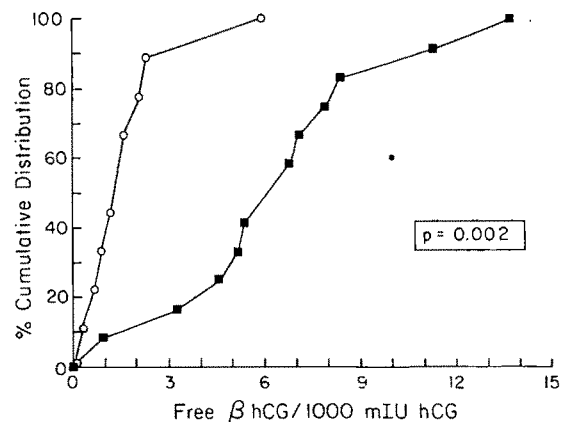


Fig. 3. Cumulative distribution ratio of nanograms of free β -hCG per 1000 mIU of hCG in serum of patients with spontaneous remission ($\circ$) and those with subsequent persistent trophoblastic disease ($\square$).

who are at high risk of developing persistent trophoblastic disease in order to justify prophylactic chemotherapy at the time of evacuation.^{4, 5, 14} These classification schemes are based on clinical parameters. The size of the uterus at the time of diagnosis, relationship of the uterine size to the date from the last menstrual period, presence or absence of variant theca-lutein cysts, and initial level of β -hCG have been used most often. In these authors' retrospective analyses the predictive accuracy rates are 39.8%, 57%, and 61.7%. These levels of accuracy are not judged sufficient to justify the use of prophylactic chemotherapy by most investigators and only one author uses the scoring system as a rationale for prophylactic chemotherapy.⁵

The results of this study are clearly preliminary because of its retrospective nature and modest patient numbers. We could find no statistical correlation between the ratio of free β -hCG to total β -hCG and the interval between uterine evacuation and date of serum sample (a few days to a few weeks) in these 21 patients, but a prospective study should provide data on the disappearance curve of free and total hCG after uterine evacuation in both patients with eventual regression and those with progressive disease. This would allow delineation of the optimal timing of plasma sampling for discrimination of high- and low-risk patients.

Despite the retrospective nature of this study, the preliminary data are very encouraging with a high percentage of patients with eventual progression being identified and a true positive rate of about 90%. A prospective study to gather large numbers of patients, standardize serum sampling times, and characterize free and total β -hCG disappearance curves is underway in the Gynecologic Oncology Group. If these preliminary results can be confirmed, prophylactic therapy can

be reevaluated with this assay to select high-risk patients and eventually change the clinical care of patients with molar pregnancy.

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Methotrexate inhibition of normal trophoblasts in vitro

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Methotrexate is a folic acid inhibitor useful in the treatment of gestational trophoblastic disease. Its effect on normal trophoblast in vitro was studied to help establish a minimum effective dose in vivo for the conservative treatment of ectopic pregnancy. In vitro inhibition of normal trophoblastic cell growth was measured after 48 hours of exposure to variable concentrations of methotrexate (10^{-2} to 10^{-9} mol/L) and rescue with leucovorin. A modification of Côté's method of trophoblastic cell enrichment was used. Long-term (10 days) linear growth inhibition was observed at concentrations $>10^{-5}$ mol/L. Leucovorin control cultures showed no inhibition of cell growth. The study demonstrates that methotrexate is effective in inhibiting normal trophoblastic cell culture growth in vitro at concentrations 1000 times those reported necessary to inhibit deoxyribonucleic acid synthesis in choriocarcinoma cell cultures. (*AM J OBSTET GYNECOL* 1986;155:324-9.)

Key words: Methotrexate, trophoblast, tissue culture, and hCG

The drug methotrexate (4-amino-4-deoxy- N^{10} -methylfolic acid) is a tetrahydrofolate reductase inhibitor that blocks the formation of reduced folates nec-

essary for the transfer of one-carbon molecules. They are important in the biosynthesis of thymidylic acid and inosinic acid. Methotrexate, while S-phase specific in its inhibition of deoxyribonucleic acid synthesis, also acts throughout the cell cycle to inhibit amino acid interconversions. Its effects in vivo and in vitro on malignant trophoblasts have been studied extensively; however, no data are available regarding the effect of methotrexate on normal trophoblasts in vitro.

In vitro effects of methotrexate on choriocarcinoma

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cells include numerous morphologic changes. Several authors¹⁻³ have reported increased nuclear and cell sizes, increased secretory granules and cell vacuoles, increased microvilli with decreased cell ruffles and filopodia, and relatively increased numbers of multinucleated cells in culture. Physiologic changes noted include an inhibition of culture growth at 10^{-6} mol/L methotrexate, an acute increase in human chorionic gonadotropin (hCG), moderate inhibition of protein synthesis, inhibition of deoxyribonucleic acid synthesis and blockage of cytokinesis with continued nuclear division.⁴ Data from Koide et al.⁵ using normal trophoblast in hamster cheek preparations suggest a similar inhibition of growth and a concomitant decrease in hCG with methotrexate treatment at 7 days.

We studied the effects of methotrexate on normal trophoblast in vitro to gain insight into the minimum effective dose for in vivo use. This project is a part of a pilot study for the treatment of unruptured ectopic pregnancy with methotrexate, as proposed by Tanaka et al.⁶

Material and methods

Media and solutions. Ham's F10 nutrient medium was used throughout for isolation and culture. Differing amounts of fetal calf serum were added, as specified in the procedures below. For processing and culture 10% fetal calf serum was used and for sedimentation 40% fetal calf serum was used. Gentamicin, 100 μ g/ml, was added unless otherwise specified. Hanks balanced salt solution, calcium and magnesium free, with 200 μ g/ml of gentamicin was used for rinsing tissue. Difco trypsin was used to digest placental tissue. All biologic supplies were purchased from Grand Island Biological Company. Methotrexate and leucovorin calcium injection were purchased from Lederle Laboratories. Methotrexate was reconstituted with sterile water according to package instructions. Leucovorin calcium was provided in a 3 mg/ml concentration.

Trophoblast cell isolation (enrichment). Placental specimens were obtained in a sterile fashion from normal abortuses of 8 to 11 weeks' gestation confirmed by ultrasonography and pathologic examination. Villi were dissected sharply from the surrounding connective tissue in calcium- and magnesium-free Hanks balanced salt solution. A modification of the method of Cotte et al.⁷ of trophoblastic cell isolation was used. Blood and debris were removed from the villi with several rinses of calcium- and magnesium-free Hanks balanced salt solution. Excess calcium- and magnesium-free Hanks balanced salt solution was removed. The villi were minced into pieces of less than 2 mm³ and placed in a sterile 2-ounce screw capped jar with 11.5 ml of 0.25% Difco trypsin. The tightly capped jar was placed in a very slowly rotating shaker bath at 37° C for 1 hour. Then 20 ml of F10-Ham's 10% fetal

calf serum was added to the jar and the tissue dissociated by 15 minutes of vigorous pipetting. The suspension was strained with a 40-mesh Bellco tissue sieve and rinsed with 11.5 ml of Ham's F10-10% fetal calf serum. The straining and rinsing were repeated. All filtrate was transferred to a sterile 50 ml screw-capped conical centrifuge tube and centrifuged at 1200 rpm for 10 minutes. The supernate was removed by suction, the pellet resuspended by 11.5 ml of Ham's F10-10% fetal calf serum, and the suspension slowly layered onto a column of 11.5 ml of Ham's F10-40% fetal calf serum in a 16 by 150 mm glass culture tube. After 90 minutes of sedimentation at room temperature the bottom 10 ml of the column was removed, diluted to 30 ml with Ham's F10-10% fetal calf serum in a 50 ml plastic screw-capped conical centrifuge tube, and then centrifuged at 1200 rpm for 8 minutes. The supernate was removed by suction and the pellet resuspended in 15 ml of Ham's F10-10% fetal calf serum.

Cell culture and treatment. One milliliter of cell suspension was randomly plated into three wells of each Lux 8 well multiplate and the test formula assigned randomly to the tray as a whole. Three milliliters of the appropriate culture medium was added to each well. Concentrations of methotrexate tested ranged from 10^{-2} to 10^{-9} mol/L. Cells were observed with a Zeiss Invertoscope D inverted phase microscope to verify even distribution over the entire culture well. The culture plates were incubated in a 37° C moist incubator in 5% carbon dioxide in air. After 48 hours the wells were rinsed with 5 ml of normal saline solution and leucovorin (10^{-1} times the dose of methotrexate in milligrams per 100 ml, in Ham's F10-10% fetal calf serum) was added to each well. Six wells were used as controls in each series of experiments. Three of these wells underwent media changes but received no methotrexate or leucovorin. The other three wells were converted to "leucovorin controls" after 48 hours by the addition of Ham's F10-10% fetal calf serum plus leucovorin at a concentration equal to 20% of the highest concentration used in the batch. Thereafter, medium, consisting of Ham's F10-10% fetal calf serum with gentamicin, was changed every 48 hours until termination at 10 days. Cultures were observed daily and terminated by fixation at 10 days.

Morphologic analysis All cultures were reviewed daily, and observations were recorded. A Zeiss Invertoscope D with attached camera was used to photograph unfixed cultures with Kodak Kodacolor ASA 100 and Kodak 160 ASA tungsten slide films.

hCG assay. Media from selected plates at 10 days of culture were retained and refrigerated. Assay for the β -subunit of hCG was performed by the John I. Brewer Trophoblastic Disease Center with the use of methods described elsewhere.⁸

hCG staining. Cells were fixed in situ as described

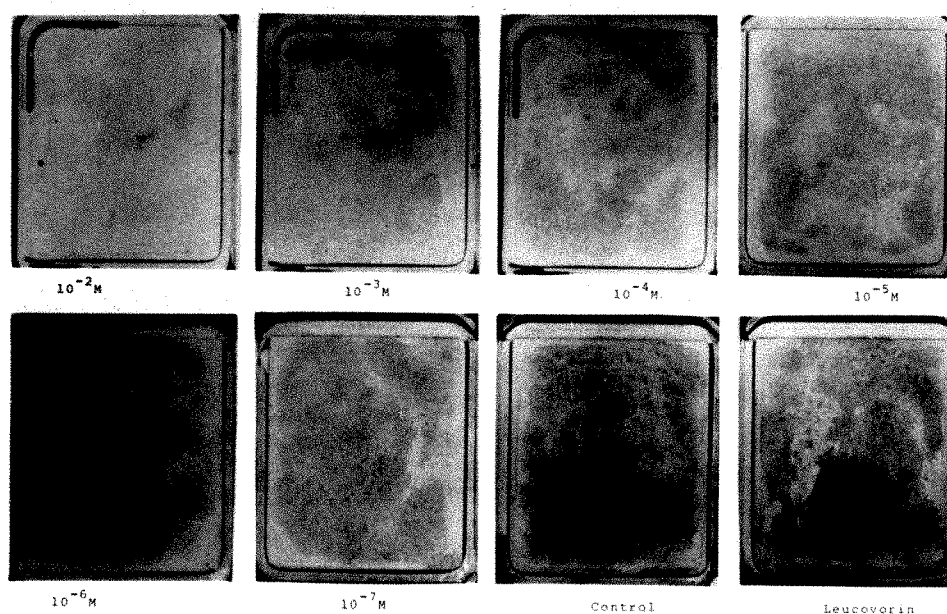


Fig. 1. Greater than 75% inhibition of growth is noted for 10^{-2} and 10^{-3} mol/L methotrexate.

Table I. Inhibition of trophoblastic cell growth with methotrexate

Methotrexate concentration (mol/L)	Growth		Significance	β -Subunit hCG* (% of control)
	% of control	Range (%)		
10^{-2} (n = 12)	3	1-6	$p < 0.005$	13
10^{-3} (n = 12)	18	12-28	$p < 0.005$	48
10^{-4} (n = 18)	28	25-38	$p < 0.005$	70
10^{-5} (n = 9)	36	32-50	$p < 0.005$	74
10^{-6} (n = 9)	48	40-70	NS	—
10^{-7} (n = 6)	52	45-62	NS	—
10^{-8} (n = 6)	78	65-85	—	—

*Pooled sample of six trials for 10^{-2} to 10^{-5} mol/L methotrexate.

by Ham and McKeehan,⁹ including a deionized water rinse, 20-minute fixation with 10% formalin, and air drying. Indirect immunoperoxidase staining with anti-hCG was performed on all plates with the method of Rurman et al.¹⁰ Fibroblast controls were also stained.

Counterstaining. After hCG staining, microscopic analysis, and photographic documentation, all culture plates were counterstained with Wright's stain. Cultures were observed for growth inhibition relative to controls and the results were photographically documented (Fig. 1). Growth was measured relative to the control cultures by the use of a 100-box graph paper overlay.

Electron microscopy. Specimens were harvested by rinsing in phosphate-buffered saline solution at 37°C followed by fixation in 2% glutaraldehyde-paraaldehyde. Fixed cells were treated and embedded as described by Anderson.¹¹ They were examined with a

Phillips PSEM-500 scanning electron microscope and photographed with Polaroid Land film, type 55.

Statistical analysis was accomplished with the Kruskal-Wallis test. This test is a nonparametric one-way analysis of variance.

Results

Significant growth inhibition (>75%) was noted for all cultures treated with 10^{-2} or 10^{-3} mol/L methotrexate (Table I). Linear inhibition of growth was found with increasing methotrexate concentration. This inhibition was significant down to concentrations of 10^{-5} mol/L ($p < 0.005$). This was confirmed by β -subunit hCG assay (Table I).

In addition to the methotrexate inhibition of growth, the numerous morphologic changes observed were similar to those described for methotrexate effects on choriocarcinoma in vitro. Cell size was inversely proportional to confluency and related to the morphologic form of the cell.

We noted four different cell forms: multiangular, spindle, oval, and "fried egg." The multiangular cells were predominantly found in open areas and were frequently multinucleated (Fig. 2). These cells were larger than the others and had numerous cell projections, which gave them the appearance of active growth. Spindle cells largely replaced the multiangular forms as confluency was approached. They would often pack in parallel bundles but could be found in small clusters in open areas. Oval cells were found in the dense growth areas and often formed mosaic patterns. Oval cells also showed multilayered growth forming what have been labeled "domes."¹² Cell borders were often indistinct in

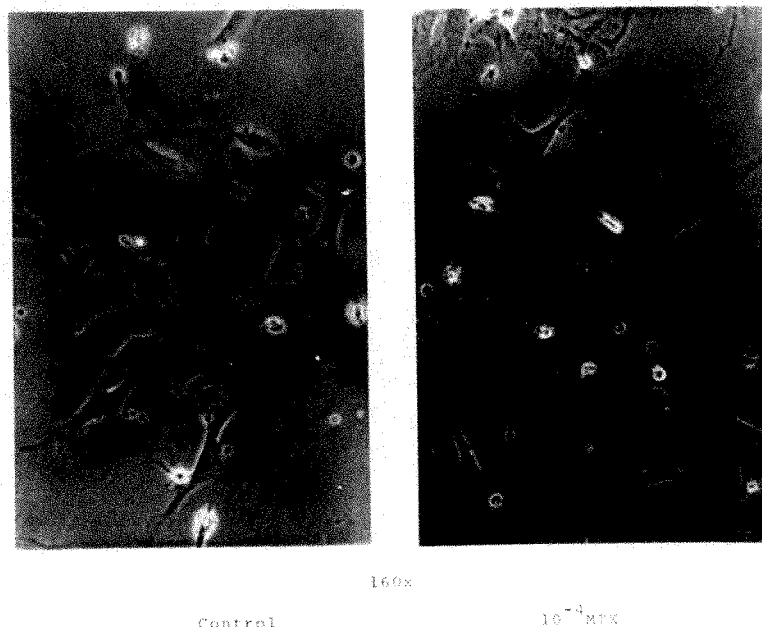


Fig. 2. In open areas mostly multiangular cells are seen in both methotrexate and control cultures.

these dense growth areas giving them the appearance of a syncytium. The "fried egg" cells were also found in the dense growth areas and were similar to those cells described by Cotte et al.⁷ as having an amorphous cytoplasm and a central nucleus. The four cell forms noted appeared as a continuum in cell culture, rather than as four different types of cells as had been previously described.¹² In confluent areas only oval and "fried egg" cell forms were noted, with the "fried egg" forms being observed only beyond 7 days of culture growth (Fig. 3). β -subunit hCG secretion was documented for these two cell forms in our study and others.^{5, 7, 12} All four cell forms stained with anti-hCG in all treatment groups except the 10^{-2} mol/L methotrexate cultures. Only 25% of the 10^{-2} mol/L methotrexate cells stained with anti-hCG whereas 90% of the cells in the remaining cultures were positive for hCG staining. hCG staining of long-term cultures was positive at 14 days but absent at 25 days.

There were other microscopically noted morphologic differences between the methotrexate-treated and the control cultures. At 7 to 10 days in culture secretory granules were more prevalent in the methotrexate-treated cells and in multinucleated cells in general when compared with the control cells. Multinucleated cells were far more prevalent in the methotrexate-treated cultures, which may account for this difference in secretory granules. Evidence of active secretion by these cells was observed by electron microscopy. Control cell populations demonstrated more evidence of growth in the nonconfluent areas when compared with the methotrexate cultures, as shown in Fig. 4. The untreated

control cells showed increased fine cell projections in the non-confluent areas. No differences were noted in cell projections in the confluent areas.

Comment

The effect of methotrexate on normal trophoblast in vitro has not been studied previously. Assessment of growth inhibition and morphologic changes was made for concentrations of methotrexate that are similar to the range used in vivo. The response of normal trophoblast to methotrexate was different from that reported for choriocarcinoma in vitro.

Methotrexate inhibits deoxyribonucleic acid formation and further choriocarcinoma cell culture growth at a threshold of 10^{-6} mol/L with an associated increase in hCG that lasts 48 hours after exposure.⁴ The inhibition of growth is also temporary, showing reversal 48 hours after exposure to methotrexate. When compared with controls, normal trophoblastic cell culture growth was inhibited by more than 75% at methotrexate concentrations of $\geq 10^{-3}$ mol/L at 10 days. hCG assay data (Table I) show 50% inhibition at the 10^{-3} mol/L methotrexate level. While these represent two different end points, it should be noted that this threshold for significant long-term growth inhibition (10^{-3} mol/L) is one thousand times greater than that reported for the inhibition of deoxyribonucleic acid formation in choriocarcinoma.⁴ Partial inhibition of growth was found at concentrations as low as 10^{-5} mol/L methotrexate. These results are similar to those of Koide et al.⁵ for normal trophoblast in hamster cheek pouch transplants treated with methotrexate. They observed both de-

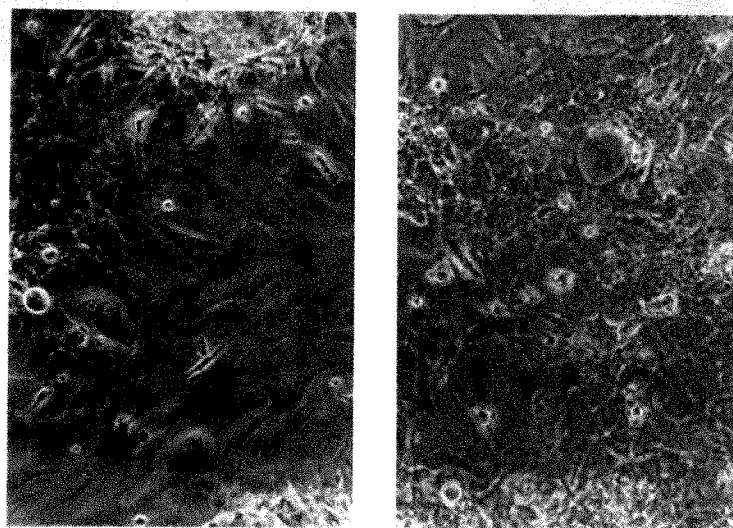
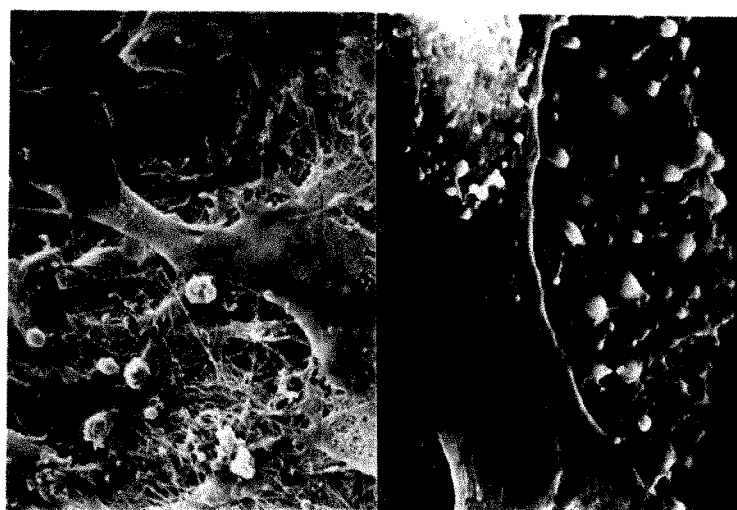


Fig. 3. In confluent areas multiangular cells are replaced by spindle cells, round cells, and "fried egg" cells regardless of treatment group.



Control 10^{-4} Mtx

Fig. 4. Control cells showed electron microscopic evidence of growth while methotrexate cells showed secretory morphologic features.

creased cell number and decreased hCG concentrations at 7 days after methotrexate treatment.

Studies of methotrexate inhibition of choriocarcinoma have shown an increase in cell and nuclear size.¹⁻³ Some of these authors have suggested that increased cell size is secondary to a methotrexate-induced halt of cytokinesis while ribonucleic acid translation, possibly somewhat inhibited, continues, thus allowing increased cytoplasmic volume. Nuclear size also increases despite methotrexate inhibition of deoxyribonucleic acid synthesis. This study also noted increases in cell and nuclear size in normal trophoblast but only

at concentrations of 10^{-2} mol/L. Below this threshold, cell size varied with confluency and multinucleation. In our study multinucleated cells were more common in methotrexate-treated cultures. As the methotrexate concentrations of the cultures increased, so did the relative percentage of multinucleated cells. Friedman and Skehan¹ found that these multinucleated cells made up <4% of control populations versus 85% in methotrexate-treated cultures. Many^{7, 13} suggest that this is an endomitotic or amitotic process whereas others¹⁴ have suggested cell fusion. The indirect effect of this multinucleation, found at the higher methotrexate concen-

trations, is an increase in secretory granules and nuclear size in many cells. Higher concentrations of methotrexate also lead to growth inhibition, decreased confluency, and larger cells.

Four populations of cell forms in vitro were identified. We labeled these multiangular cells (consistent with Loke's "crazy pavement cells") as spindle cells, oval cells, and "fried egg" cells. All four forms stained with the anti-hCG indirect immunoperoxidase method. The oval forms predominate at 10 days in culture and have been described by Loke¹² and Cotte et al.⁷ as cytotrophoblast because of hCG synthesis demonstrated by immunofluorescence and immunoperoxidase staining. Spindle and multiangular forms have not been shown to be secretors of hCG in the past and were felt to represent fibroblasts, macrophages, and decidual cells. This inability to demonstrate hCG staining of multiangular and spindle cells by previous investigators may be due to the immaturity of cells early in culture life. As Cotte et al.⁷ have shown, cytotrophoblasts did not stain with anti-hCG until after 48 hours in culture. Maturation into multi-nucleated secretory forms must first occur. This need for maturation is also suggested in this investigation. Only two cell forms were noted within the first 48 hours: multiangular forms in open areas occurring as single cells and oval forms occurring in clusters. As confluency was approached the multiangular forms were largely replaced by spindles and more oval forms. At 7 to 10 days spindle forms were largely replaced by ovals and the rare "fried egg" cells.

The fact that all four cell forms stained with anti-hCG immunoperoxidase techniques suggests that all are trophoblasts. The continuum represented by these cell forms suggests that these shapes may represent the maturation of single trophoblast cells in vitro rather than three or four different types of cells, as they respond to their culture conditions. Further work with time-elapse photography and radioisotope labeling is necessary to document this process. Morphologic identification of trophoblast in vitro is frequently misleading and should be confirmed by hCG data. Cell identification should not be made on the basis of appearances similar to those of in vivo tissue. Such parallels have not been demonstrated. Cell morphologic features in vitro vary widely when compared with those in vivo and are extremely dependent on culture conditions. A

syncytial tissue such as trophoblast is even less likely to maintain its three-dimensional structure in vitro.

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T-cell subsets and natural killer cell activity in patients with gestational trophoblastic neoplasia

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Lymphocyte counts, T-cell counts, B-cell counts, helper T-cell counts, and suppressor-cytotoxic T-cell counts were performed in 38 patients with gestational trophoblastic neoplasia and 38 normal control subjects. Natural killer cell activity was also assayed in 30 patients and 30 control subjects. The percentage and count of lymphocytes and the absolute counts (but not the percentages) of T cells, helper T cells, and suppressor-cytotoxic T cells in patients with gestational trophoblastic neoplasia were significantly lower than those of normal control subjects. In high-risk patients with gestational trophoblastic neoplasia, there was also a significant reduction of the helper T cell to suppressor-cytotoxic T-cell ratio in comparison with that in normal control subjects. There was no significant difference in the B-cell counts or natural killer cell activity. The measurement of these parameters was not useful in predicting the response to chemotherapy. (AM J OBSTET GYNECOL 1986;155:330-4.)

Key words: T cells, natural killer cells, gestational trophoblastic tumors

With the availability of the various monoclonal antibodies directed against T-cell surface epitopes, the T-cell subsets in various physiologic and pathologic conditions can readily be studied. During pregnancy, there is a significant decrease in both the percentage and the number of helper T cells while the percentage and number of suppressor-cytotoxic T cells are not affected.¹ In patients with gestational trophoblastic neoplasia, we have found that the absolute counts of lymphocytes, B cells, T cells, and T cells bearing Fc receptors for IgG were significantly lower than in normal control subjects.^{2,3} However, the T cells bearing Fc receptors for IgG are not a homogeneous population of suppressor cells⁴ and monoclonal antibodies provide better definition of both helper T cells and suppressor-cytotoxic T cells.

Natural killer cells constitute a population of non-sensitized non-T, non-B lymphocytes capable of in vitro cytotoxicity against tumor target cells. It is believed that the natural killer cells may have the ability to mediate natural resistance against tumors in vivo and so play an important role in immune surveillance. Suppression of natural killer cell activity has been reported in pregnancy⁵ and various forms of malignancies.⁶ In vitro experiments have also demonstrated that the natural

killer cell activity was suppressed by the presence of human chorionic gonadotropin (hCG).⁷ Gestational trophoblastic neoplasia is an hCG-secreting tumor; therefore, the natural killer cell activity may also be affected in that disease. In view of these previous findings we studied the T-cell subsets and the natural killer cell activity in patients with gestational trophoblastic neoplasia and assessed the prognostic value of these parameters in predicting the response to chemotherapy.

Patients and methods

Patients admitted with a diagnosis of persistent gestational trophoblastic neoplasia or choriocarcinoma were studied. Our criteria for diagnosing postmolar persistent gestational trophoblastic neoplasia have been reported before.⁸ Briefly, after evacuation of hydatidiform moles, patients in whom the serum levels of the β -subunit of hCG (β -hCG) continued to rise for more than 3 consecutive weeks and patients with metastasis and elevated serum β -hCG levels were treated with chemotherapy. The patients were classified into low-risk and high-risk groups by a scoring system⁹ modified from that of Bagshawe.¹⁰ Low-risk patients were treated with oral etoposide.⁹ High-risk patients were treated with the CHAMOMA (Cytosan, hydroxyurea, actinomycin D, methotrexate, Oncovin, melphalan, and Adriamycin) regimen.¹¹ Blood was taken for immunologic studies before chemotherapy was started. The normal control subjects were selected from healthy patients admitted for sterilization and also from volunteers among our own staff.

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Table I. Clinical data of patients studied

	<i>Low risk</i>	<i>High risk</i>	<i>Total</i>
No.	25	13	38
Age (mean $\pm$ SD) (yr)	33.2 $\pm$ 10.7	38.9 $\pm$ 13.0	35.2 $\pm$ 11.7
Serum β -hCG (mean $\pm$ SD) (IU/L)	11,751 $\pm$ 19,152	75,617 $\pm$ 158,162	33,600 $\pm$ 96,404
Antecedent pregnancy			
Hydatidiform mole	25	10	35
Abortion	0	1	1
Term delivery	0	2	2
Patients with metastasis (No.)	4 (16%)	10 (76.9%)	14 (36.8%)

The total lymphocyte count was calculated from the total leukocyte count and the leukocyte differential count.

To separate the mononuclear cells, aliquots of 10 ml of heparinized blood from the patients or the normal control subjects were layered onto 3.5 ml of Ficoll-Hypaque (specific gravity = 1.077) and centrifuged at $800 \times g$ for 15 minutes. The mononuclear cells were harvested from the interface and washed three times in Hanks balanced salt solution. They were resuspended in Hanks balanced salt solution and cell concentrations were adjusted to appropriate levels for cell marker studies.

The T-cell count was performed by the E-rosette method.¹² B cells were identified by the presence of surface immunoglobulins and they were counted as described previously.³

T-cell subsets were studied by an indirect immunofluorescent technique with the use of murine monoclonal antibodies OKT4 (against helper T cells), OKT8 (against suppressor-cytotoxic T cells), and OKT11 (against all peripheral T cells), from Ortho Pharmaceuticals. The mononuclear cell suspension, 0.1 ml at a concentration of 8.0×10^6 cells per milliliter, was added to 5 μ l of the appropriate antisera (OKT4, OKT8, or OKT11). After incubation at 4° C for 30 minutes, all cells were washed twice with phosphate-buffered saline containing 0.05% sodium azide and 1% bovine serum albumin. Then 50 μ l of fluorescein isothiocyanate-conjugated antimouse IgG (diluted 1:10) was added and incubated at 4° C for 30 minutes. The cells were washed three times with phosphate-buffered saline, resuspended in a drop of FA mounting fluid (Difco), and read under an ultraviolet microscope.

Natural killer cell activity was assayed against the target cell line K562. The target cells, 10^6 cells in 0.2 ml of RPMI medium, were labelled with 100 μ Ci of chromium 51 (1 mCi/ml) by incubation at 37° C for 1½ hours. The target cells were then washed twice in RPMI medium supplemented with 10% fetal calf serum and the concentration was adjusted to 10^5 /ml. The mononuclear cells were adjusted to a concentration of 8×10^6 /ml and diluted serially at twofold to 0.5×10^6 /ml. Aliquots of 0.1 ml of lymphocyte suspensions at

these five concentrations were dispensed into the wells of a round-bottomed micro T/C tray (Nunc), and 0.1 ml of target cells was added to each well. Therefore, the target-effector cell ratios studied were 1:80, 1:40, 1:20, 1:10, and 1:5. Assays were done in quadruplicate. The spontaneous release was assayed by incubating 0.1 ml of RPMI medium with 0.1 ml of target cells and the total releasable ⁵¹Cr assayed by addition of 0.1 ml of NP40 (1%) to 0.1 ml of target cells. The whole plate was incubated at 37° C in 5% carbon dioxide for 3½ hours. After incubation, the plate was centrifuged at $400 \times g$ for 10 minutes. Aliquots of the supernatant fluid (0.1 ml) were dispensed into the counting tubes and counted in a gamma counter. The specific cytotoxicity at the different target-effector ratios was calculated by the formula:

$$\% \text{ Cytotoxicity} = 100 \times \frac{^{51}\text{Cr released by effectors} - ^{51}\text{Cr release in medium}}{^{51}\text{Cr released by detergent} - ^{51}\text{Cr release in medium}}$$

The β -hCG was assayed by radioimmunoassay as described by Vaitukaitis et al.¹³ The purified β -hCG for radioiodination and the antibody against β -hCG were supplied by the National Institutes of Health.

Differences between the means were analyzed by Student's *t* test.

Results

Thirty-eight patients with gestational trophoblastic neoplasia and 38 normal control subjects were studied. The clinical data of the patients are shown in Table I. One low-risk patient showed resistance to oral etoposide but subsequently responded to the CHAMOMA regimen. Four patients in the high-risk group did not respond to the CHAMOMA regimen. Two of these patients subsequently died despite treatment with other drugs. One patient responded to other forms of chemotherapy and another patient was still receiving treatment at the time of writing of this report.

The results of the peripheral blood leukocyte counts, lymphocyte counts, B-cell counts, and T-cell counts (by the E-rosette method) are shown in Table II. The percentage of lymphocytes, the absolute lymphocyte

Table II. Peripheral blood counts of leukocytes, lymphocytes, T cells, and B cells in gestational trophoblastic neoplasia and normal control subjects

	Gestational trophoblastic neoplasia			Normal control subjects (n = 38)
	Low risk (n = 25)	High risk (n = 13)	Total (n = 38)	
White blood cell count ($10^9/L$)	6.77 $\pm$ 1.56	7.30 $\pm$ 2.19	6.95 $\pm$ 1.79	6.83 $\pm$ 1.83
Lymphocytes (%)	22.32 $\pm$ 8.84	17.23 $\pm$ 7.74	20.58 $\pm$ 8.73	29.39 $\pm$ 7.64
Lymphocyte count ($10^9/L$)	1.47 $\pm$ 0.64	1.19 $\pm$ 0.60	1.38 $\pm$ 0.63	1.97 $\pm$ 0.64
T cells (%)	75.50 $\pm$ 8.18	78.54 $\pm$ 9.32	76.54 $\pm$ 8.58	76.61 $\pm$ 8.78
T-cell count ($10^9/L$)	1.11 $\pm$ 0.52	0.84 $\pm$ 0.52	1.05 $\pm$ 0.52	1.51 $\pm$ 0.53
B cells (%)	13.12 $\pm$ 5.60	13.69 $\pm$ 6.36	13.32 $\pm$ 5.79	11.26 $\pm$ 4.40
B-cell count ($10^9/L$)	0.19 $\pm$ 0.10	0.16 $\pm$ 0.10	0.18 $\pm$ 0.10	0.22 $\pm$ 0.11

Results are expressed as means $\pm$ SD.**Table III.** T cells and T-cell subsets in gestational trophoblastic neoplasia and normal control subjects

	Gestational trophoblastic neoplasia			Normal control subjects (n = 38)
	Low risk (n = 25)	High risk (n = 13)	Total (n = 38)	
OKT11 ⁺ cells (%)	82.20 $\pm$ 11.73	82.23 $\pm$ 8.19	82.21 $\pm$ 10.53	81.87 $\pm$ 8.32
OKT11 ⁺ cell count ($10^9/L$)	1.22 $\pm$ 0.60	0.99 $\pm$ 0.55	1.14 $\pm$ 0.59	1.60 $\pm$ 0.52
OKT4 ⁺ cells (%)	53.96 $\pm$ 10.06	48.85 $\pm$ 11.09	52.21 $\pm$ 10.56	53.18 $\pm$ 7.7
OKT4 ⁺ cell count ($10^9/L$)	0.79 $\pm$ 0.37	0.59 $\pm$ 0.37	0.72 $\pm$ 0.38	1.04 $\pm$ 0.35
OKT8 ⁺ cells (%)	33.28 $\pm$ 10.52	37.00 $\pm$ 8.94	34.55 $\pm$ 10.04	33.16 $\pm$ 8.5
OKT8 ⁺ cell count ($10^9/L$)	0.49 $\pm$ 0.28	0.45 $\pm$ 0.26	0.48 $\pm$ 0.27	0.67 $\pm$ 0.31
T _H /T _S ratio	1.81 $\pm$ 0.83	1.36 $\pm$ 0.35*	1.65 $\pm$ 0.73	1.70 $\pm$ 0.49*

Results are expressed as means $\pm$ SD.*Difference is statistically significant ($p < 0.05$).

counts, and the T-cell counts in patients with gestational trophoblastic neoplasia were significantly lower than those in normal control subjects, but the difference in other parameters was not statistically significant. There was no significant difference between the high-risk patients and low-risk patients in any of these parameters.

The blood counts of T cells (OKT11⁺) and T-cell subsets are shown in Table III. The absolute counts but not the percentages of OKT11⁺, OKT4⁺, and OKT8⁺ cells in patients with gestational trophoblastic neoplasia were significantly lower than those in normal control subjects. The OKT4/OKT8 ratio in normal control subjects was significantly higher than that in high-risk patients but it was not significantly different from that in low-risk patients or in gestational trophoblastic neoplasia as a whole. Although the absolute counts of OKT11⁺, OKT4⁺, and OKT8⁺ cells and the OKT4/OKT8 ratio were all lower in high-risk patients than in low-risk ones, these differences did not reach levels of statistical significance.

There was, as expected, a significant correlation be-

tween the T-cell count by the E-rosette method and the T-cell count by the OKT11 monoclonal antibody method ($r = 0.46$, $p < 0.001$); OKT11 is believed to define the E-rosette receptor.

The natural killer cell assay was performed in 30 patients, 20 in the low-risk group and 10 in the high-risk group. The results are shown in Table IV. At all target-effector cell ratios there was no significant difference in cytotoxicity between patients with gestational trophoblastic neoplasia and normal control subjects. There was also no significant difference between the high-risk group and the low-risk group at all the target-effector ratios. The correlation between the serum β -hCG levels (after logarithmic conversion because the distribution was skewed) and the natural killer cell activity in gestational trophoblastic neoplasia was also analyzed, but there was no significant correlation at any of the target-effector cell ratios. The correlation coefficients were 0.21, 0.24, 0.24, 0.15, and 0.08 at target-effector cell ratios of 1:5, 1:10, 1:20, 1:40, and 1:80, respectively ($p > 0.05$ for all).

The results of these tests in the five patients with

Table IV. Natural killer cell cytotoxic activity in gestational trophoblastic neoplasia and normal control subjects

Target-effector cell ratio	Gestational trophoblastic neoplasia			Normal control subjects (n = 30)
	Low risk (n = 20)	High risk (n = 10)	Total (n = 30)	
1 : 5	20.5 ± 11.9	23.9 ± 11.5	21.7 ± 11.7	23.7 ± 11.3
1 : 10	28.4 ± 13.5	36.2 ± 13.5	31.0 ± 13.8	33.9 ± 13.5
1 : 20	38.0 ± 14.6	45.6 ± 14.4	40.5 ± 14.7	44.6 ± 16.4
1 : 40	47.2 ± 13.8	57.1 ± 13.9	50.5 ± 14.4	54.1 ± 15.9
1 : 80	54.1 ± 12.0	61.8 ± 12.2	56.7 ± 12.4	62.0 ± 13.5

Results are expressed as means ± SD of specific cytotoxicity.

resistance to chemotherapy were similar to those in patients with good response to chemotherapy.

Comment

The results of this study confirmed previous findings that the percentage and absolute number of lymphocytes and T cells in the peripheral blood in patients with gestational trophoblastic neoplasia were significantly lower than those in normal control subjects. The decrease in absolute counts affected both T_1 and T_8 , the principal subclasses of T cells. There was a significant change in the ratio of helper T cells to suppressor-cytotoxic T cells only in high-risk patients. Thus in gestational trophoblastic neoplasia there is a decrease in all the subclasses of T cells without any significant change in T_4/T_8 , unless the disease is more advanced, when a decrease in T_4/T_8 is observed. However, it should be noted that the mean T_4/T_8 ratio in high-risk patients was still within the normal range.

In a previous study² with the use of the EAC rosette method, the B-cell counts in patients with gestational trophoblastic neoplasia were significantly lower than those in normal control subjects. This differs from our results in this study with the presence of surface immunoglobulin used to identify B cells. Since the EAC-rosetting cells include a population of non-T, non-B cells with complement receptors on their surfaces, the results of our present study are likely to reflect B-cell counts more accurately.

The natural killer cell activity in patients with gestational trophoblastic neoplasia was not significantly different from that in normal control subjects at any of the target-effector cell ratios. Studies in other forms of malignancies have shown that the natural killer cell activity is suppressed only in advanced stages of the disease.⁶ In gestational trophoblastic neoplasia, the serum β -hCG levels reflected the tumor burden in the patients. Nevertheless there was no correlation between the natural killer cell activity and the serum β -hCG level. There was also no significant difference in the natural killer cell activity between the high-risk group and the low-risk group although patients in the former

group had higher serum β -hCG levels and more advanced disease.

The sera in patients with gestational trophoblastic neoplasia have been shown to suppress the lymphocyte response to mitogen stimulation,¹¹ the lymphocyte cytotoxic action to the BeWo cell line,¹⁵ and the lymphocyte response to paternal alloantigens in the mixed lymphocyte reaction.¹⁶ Our natural killer cell assays were performed after washing of the cells, and serum inhibitory factors, if present, may have been washed off. Therefore, the results of the natural killer cell activity might be quite different if the assays were performed in the presence of the patients' sera. Studies of the effect of the patients' sera on natural killer cell activity in vitro are now in progress.

This study also showed that there was no significant difference in any of these parameters between the high-risk group and the low-risk group. The results in patients who responded to chemotherapy were also similar to those who showed resistance. Therefore, the measurement of these variables is of little value in predicting the response to chemotherapy, a finding similar to our conclusion with the use of other immunologic tests.^{2,3} Other factors,¹⁰ such as the type of antecedent pregnancy, the interval from antecedent pregnancy, the serum β -hCG levels, and the presence of metastases, are probably more important in predicting the response to chemotherapy.

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Endocrine impact of pure estradiol replacement in postmenopausal women: Alterations in anterior pituitary hormone release and circulating sex steroid hormone concentrations

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Thirteen healthy postmenopausal volunteers were studied under basal conditions and at intervals (days 1, 5, 10, and 30) after intravaginal placement of a polysiloxane ring impregnated with 400 mg of estradiol. Mean serum estradiol concentrations rose 26-fold with a twofold increase in serum estrone concentrations. Serum Δ^4 -androstenedione, dehydroepiandrosterone sulfate, and total testosterone did not change, but absolute and percent free testosterone concentrations declined significantly by day 5. Concurrently, serum concentrations of immunoreactive follicle-stimulating hormone declined progressively, while serum luteinizing hormone and free α -subunit concentrations exhibited a biphasic pattern of suppression. Serum levels of prolactin increased monophasically and those of growth hormone, somatomedin C, and thyroid-stimulating hormone did not change. (*AM J OBSTET GYNECOL* 1986;155:334-9.)

Key words: Estradiol, menopause, pituitary, gonadotropins, sex steroids

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The precise endocrine consequences of estrogen replacement in healthy postmenopausal women are poorly understood. In part, this limitation reflects the wide variations in synthetic and naturally occurring estrogen preparations used, as well as the susceptibility of orally administered steroids to undergo extensive enteric metabolism.¹⁻³ As one alternative approach, polysiloxane rings have been impregnated with pure steroid hormone and inserted intravaginally. This experimental delivery system is intended to provide a relatively steady-state release of steroid hormone into the pelvic circulation and thereby obviate the influences of enteric metabolism, portal absorption, and "bolus" surges of circulating estrogen that follow oral dosing.^{4,5}

In an earlier study of an estradiol-impregnated polysiloxane vaginal ring, we documented that physiologic circulating levels of estradiol were attainable in postmenopausal women, at least after 1, 2, or 3 months of estrogen administration.⁶ At such times, serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were significantly suppressed. These promising initial studies raised the following additional experimental questions: (1) Is intravaginally administered estradiol converted to estrone as extensively as after oral administration? (2) What is the impact of this sustained mode of estradiol replacement on circulating concentrations of other sex steroid hormones? (3) What are the time course and nature of the biologic actions of pure estradiol on the secretion of individual pituitary hormones? Accordingly, in the present studies, we have used the estradiol-containing polysiloxane ring to search for discrete, prompt, and specific alterations in individual steroid and peptide hormone concentrations in response to selective estradiol replacement.

Methods

Estradiol-containing polysiloxane rings were prepared exactly as described earlier, with a dose of 400 mg of pure estradiol impregnated in each ring.⁶ Healthy postmenopausal women were studied after provision of written informed consent, approved by the Human Investigation Committee of the University of Virginia School of Medicine. The women who participated in this study ranged in age from 51 to 63 years (mean 57.1 ± 0.87 years, $N = 13$ women) and were spontaneously postmenopausal for 3.5 to 8 years (median 5 years). Each volunteer underwent a detailed history and physical examination (average weight 53 ± 1.8 kg, range 49 to 65 kg), with the documentation of normal hepatic, renal, and hematologic function, biochemical euthyroidism, and postmenopausal concentrations of gonadotropic hormones. At least 5 weeks before study, women were withdrawn from any drugs, including estrogen and sex steroids.

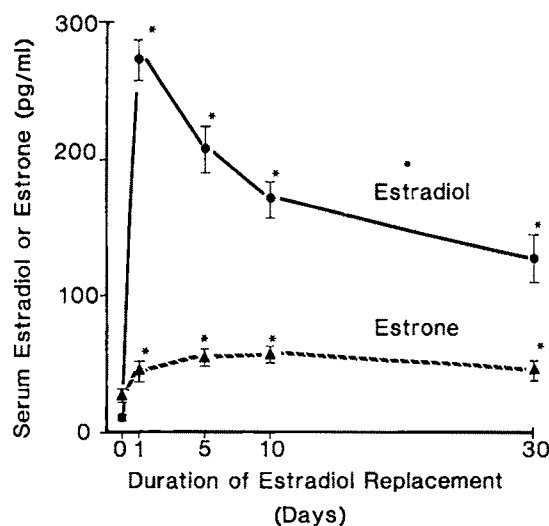


Fig. 1. Serum estradiol and estrone concentrations (means $\pm$ SEM, pg/ml) in relation to estrogen administration via a polysiloxane ring containing 400 mg of estradiol placed intravaginally. The time course of serum estradiol and estrone changes was evaluated in 13 healthy postmenopausal women under basal conditions (days 0) and on days 1, 5, 10, and 30 of treatment. The serum estradiol and estrone concentrations were measured in sera pooled from blood withdrawn at 15-minute intervals for 4 hours beginning at 8:00 AM on the indicated days of estradiol replacement. Asterisks indicate $p < 0.01$ versus basal value (before estradiol replacement).

Sampling was conducted in the Clinical Research Center by withdrawal of blood at 15-minute intervals for 4 hours beginning at 8:00 AM. Sampling was performed basally and on days 1, 5, 10, and 30 after initiation of estradiol replacement. Hormone measurements were undertaken in a pool of serum derived from aliquots of the 17 individual samples collected during a 4-hour period so that mean serum hormone concentrations might be estimated more accurately.

Serum concentrations of LH, FSH, and alpha subunit were assayed as described earlier.^{7,8} The concentrations of prolactin, growth hormone, thyroid-stimulating hormone (TSH), and somatomedin C were measured by radioimmunoassay, as reported earlier.^{9,10} In the TSH, LH, and FSH assays, the cross-reactions with α -subunit were 2%, <5%, and 0%, respectively. In the α -subunit assay, there were cross-reactions of 2.5% to 3.0% for TSH, LH, and FSH. Serum concentrations of testosterone, free testosterone, percent free testosterone, estradiol, estrone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione were also quantitated by specific radioimmunoassay after chromatography, as published previously.^{11,12} The sex steroid hormone concentrations were measured in 13 individuals, and detailed peptide hormone measurements were performed in a subgroup of nine women in whom sufficient serum was available.

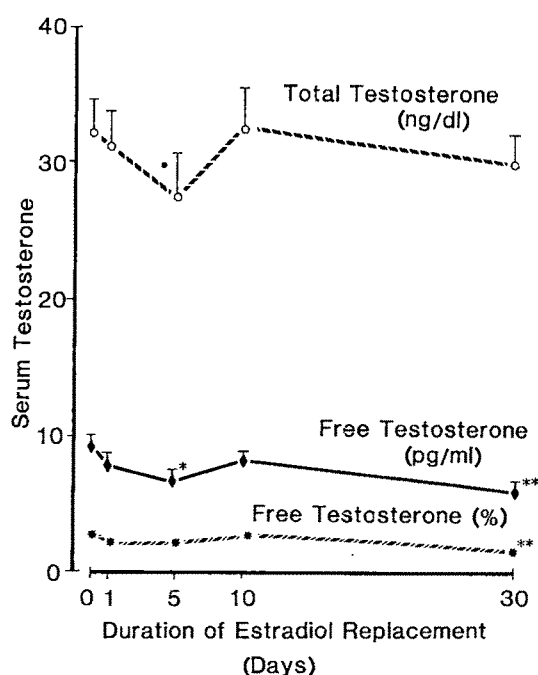


Fig. 2. Influence of estradiol replacement on mean serum total testosterone, free testosterone, and percent free testosterone concentrations in postmenopausal individuals. Data are presented as indicated in the legend of Fig. 1. * = $p < 0.05$ versus basal value. ** = $p < 0.01$ versus basal value.

The ages and clinical and biochemical characteristics of the two groups were not distinguishable.

Data are expressed as means $\pm$ SEM for the group of volunteers. Significant overall treatment effects were sought by analysis of variance with the Newman-Keuls procedure to test individual effects. Specific *a priori* comparisons were made within subjects by paired two-tailed Student's *t* testing, with Bonferroni's correction where appropriate.¹³

Results

Sex steroid hormone responses to intravaginal delivery of estradiol. As shown in Fig. 1, before estradiol treatment, basal serum concentrations of estradiol and estrone were in the normal postmenopausal range, at 10.4 ± 0.6 and 27.9 ± 4.0 pg/ml, respectively. Within 24 hours of insertion of the estradiol-containing polysiloxane ring, serum concentrations of estradiol increased significantly to peak values of 275 ± 20 pg/ml ($p < 0.001$ versus basal value). This 27-fold increase in serum estradiol concentrations was accompanied by an approximately 1.75-fold increase in serum estrone concentrations ($p < 0.01$ versus basal level), which continued to increase slightly to a peak value of 54.7 ± 3.3 pg/ml on day 10 ($p = \text{NS}$ versus day 1 value). In contrast, serum estradiol concentrations declined gradually from day 1 onward with mean values of 169 ± 13 (day

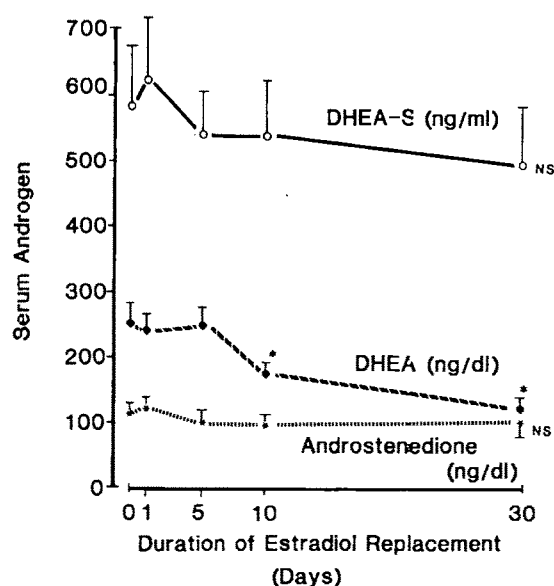


Fig. 3. Influence of estradiol replacement on mean serum concentrations of DHEA-S, DHEA, and androstenedione in postmenopausal women. Data are presented as in the legend of Fig. 1. * = $p < 0.01$ versus basal value.

10) and 126 ± 19 (day 30) pg/ml. These values were significantly above basal levels ($p < 0.001$).

In conjunction with the preferential increase in serum estradiol concentrations, serum total testosterone levels did not change significantly, although there was a slight decrease on day 5 followed by an increase on day 10 of estradiol replacement (Fig. 2). In contrast, the percent unbound testosterone declined significantly on day 5 (from a basal value of $4.2\% \pm 0.3\%$ to $3.7\% \pm 0.3\%$ on day 5, $p < 0.01$), and this decrease was also observed on day 30 (percent free testosterone 3.4 ± 0.3 , $p < 0.01$ versus basal level). The absolute serum concentrations of unbound testosterone (picograms per milliliter) also decreased significantly on day 5 and day 30 compared with baseline levels. These significant decreases in unbound testosterone concentrations presumably reflected the ability of estradiol to increase circulating concentrations of sex steroid hormone-binding globulin.

Estradiol replacement also resulted in decreased serum concentrations of DHEA, with levels of 186 ± 26 ng/dl on day 10 compared with basal values of 250 ± 36 ng/dl ($p < 0.05$), with a further decline to a mean of 120 ± 19 ng/dl on day 30 ($p < 0.01$ versus basal level) (see Fig. 3). In contrast, serum concentrations of DHEA-S and androstenedione were not significantly influenced by estradiol replacement at any time during the 30 days of observation.

Serum estrone and estradiol concentrations were not significantly correlated, except on day 1 of estrogen

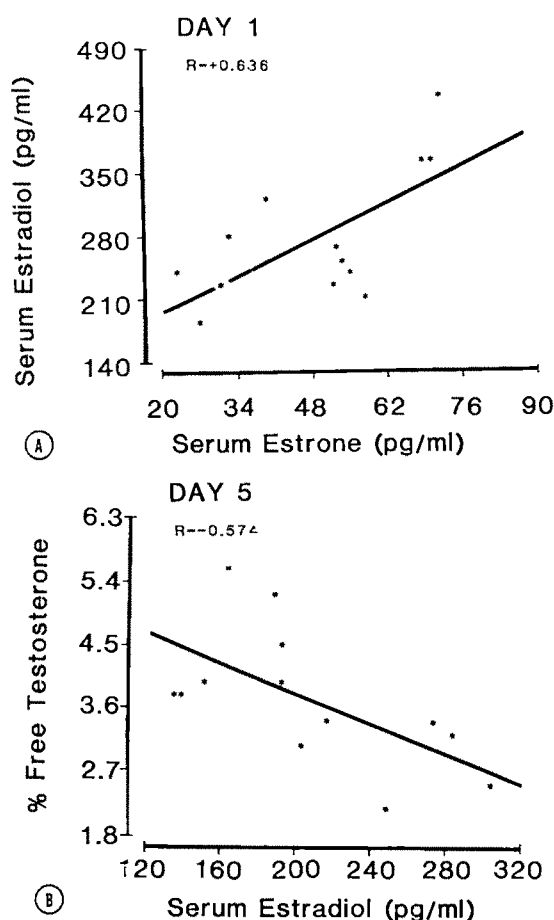


Fig. 4. A: Positive correlation between serum estrone and serum estradiol concentrations on day 1 of estradiol replacement. Data are derived from 13 healthy postmenopausal women, treated with an estradiol-containing polysiloxane ring for 24 hours. B: Negative correlation between serum estradiol and percent free testosterone concentrations on day 5 of estradiol replacement. Each asterisk represents data from an individual woman from whom blood samples were withdrawn at 15-minute intervals for 4 hours to provide a mean estimate of sex steroid hormone concentrations.

replacement: $r = 0.636$ ($p < 0.05$) (Fig. 4, A). After 5 days of estradiol replacement, the percent free testosterone was significantly inversely correlated with the serum estradiol concentration ($r = -0.574$, $p = 0.05$) (Fig. 4, B). Similarly, on day 5, serum absolute free testosterone (picograms per milliliter) was inversely correlated with serum estradiol ($r = -0.739$, $p < 0.01$). The latter observations support the view that estradiol treatment resulted in an acute increase in testosterone binding proteins with a consequent decrease in the percent and absolute concentrations of free testosterone. By day 30, serum estrogen concentrations were positively correlated with serum total testosterone concentrations: $r = 0.599$ for the correlation between serum estradiol and total testosterone and $r = 0.596$

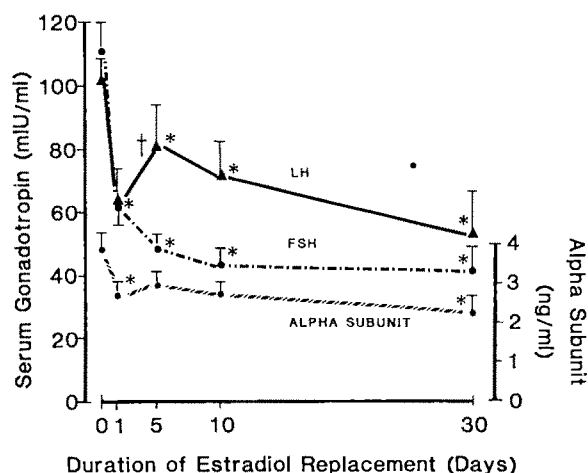


Fig. 5. Time-dependent alterations in serum gonadotropin or α -subunit concentrations in relation to estradiol replacement. Data represent mean serum gonadotropin or α -subunit concentrations at indicated intervals of estradiol replacement with a polysiloxane ring containing 400 mg of estradiol. * = $p < 0.01$ versus basal value (day 0). ** = $p < 0.05$ versus day 1 value for mean LH concentrations on day 5.

for the correlation between serum estrone and total testosterone ($p < 0.05$).

Pituitary hormone responses to intravaginal delivery of estradiol. The effect of estradiol replacement on serum concentrations of peptide hormones and glycoprotein hormones was evaluated. As shown in Fig. 5, treatment with estradiol resulted in a prompt and significant decline in serum concentrations of LH, FSH, and free α -subunit within 24 hours ($p < 0.01$). Serum FSH concentrations continued to decrease thereafter and remained suppressed during the 30 days of estradiol replacement. In contrast, on day 5 of estradiol treatment, there was a significant increase in serum concentrations of LH ($p < 0.05$ compared with the value of day 1 of estrogen replacement), followed by a gradual further suppression of LH concentrations on days 10 and 30 ($p < 0.05$ for LH concentrations on day 30 versus day 5). This triphasic pattern was also apparent in the case of α -subunit values, although the increased concentrations on day 5 were not statistically greater than those on day 1. The tendency for serum α -subunit concentrations to correlate with those of LH was statistically significant on days 5 and 30 of estrogen replacement: $r = 0.692$ and 0.665 ($p < 0.05$), respectively. Thus the pattern for FSH suppression by estradiol was distinctive, in that sustained suppression of FSH levels occurred in contrast to biphasic patterns for LH and α -subunit.

Gonadotropin responses to estradiol replacement were further distinguishable from those of other pituitary peptide and glycoprotein hormones, as shown

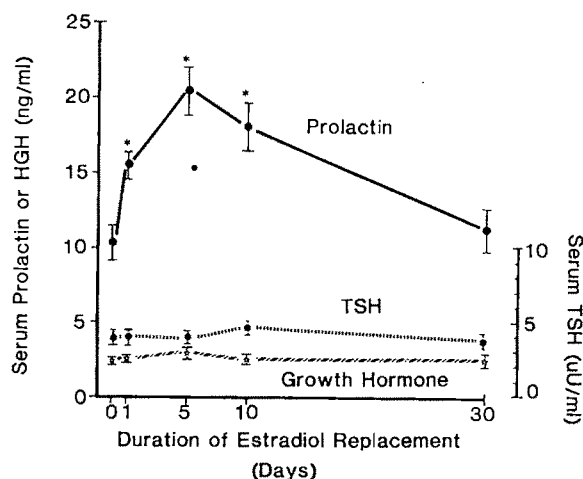


Fig. 6. Time course of mean serum prolactin, TSH, or growth hormone concentrations in response to pure estradiol replacement in postmenopausal individuals. Data are presented as in the legend of Fig. 5. * = $p < 0.01$ versus basal value.

in Figs. 5 and 6. Estradiol replacement elicited a prompt and significant increase in mean serum prolactin concentrations within 24 hours ($p < 0.01$), with a further increase after 5 days of estrogen replacement, followed by a gradual decline on day 10 ($p < 0.01$ versus basal value). Prolactin concentrations returned to basal levels by day 30. The striking increase in serum prolactin concentrations observed within 24 hours of estradiol replacement was positively correlated with serum estradiol and serum estrone concentrations at that time: $r = 0.701$ and 0.687 , respectively ($p < 0.05$).

The pattern of acutely increased prolactin concentrations after estradiol was not observed in the case of growth hormone, TSH (Fig. 6), or somatomedin C. Values of the latter growth factor were (mean $\pm$ SEM) 0.55 ± 0.05 (day 0), 0.57 ± 0.06 (day 5), and 0.52 ± 0.06 (day 30) IU/ml ($p = \text{NS}$).

Serum androgen concentrations were not significantly correlated with serum gonadotropin or prolactin concentrations at any time, except on day 5 when serum LH correlated inversely with serum free testosterone ($r = -0.746$, $p < 0.05$).

Comment

Estradiol administration via an intravaginally placed polysiloxane matrix promptly increased serum estradiol concentrations (by 26-fold within 24 hours). This increase was associated with a contrastingly small (two-fold) increase in serum estrone concentrations. This predominant increase in estradiol rather than estrone stands in distinct contrast to the pattern observed after oral estrogen administration.^{1-6, 14}

In response to estradiol replacement, mean serum concentrations of free testosterone declined signifi-

cantly within 5 days. Mean serum concentrations of DHEA also fell, but there were no major changes in serum levels of DHEA-S or androstenedione. The acute decreases in serum free testosterone (expressed as either percent unbound or absolute free hormone) presumably reflected increased blood concentrations of sex steroid hormone-binding globulin, which would be anticipated after estrogen replacement.¹⁵ On the other hand, the progressive decrease in serum concentrations of DHEA (which is sparingly bound to high-affinity plasma proteins) by days 10 and 30 of estrogen replacement may reflect alterations in metabolic clearance and/or adrenal secretion of this androgen. Available data do not allow us to distinguish between these two possible mechanisms.

Delivery of premenopausal concentrations of estradiol also resulted in distinctive time-dependent changes in mean serum concentrations of pituitary hormones. Serum LH concentrations exhibited a triphasic response to estradiol: (1) initial suppression within 24 hours, (2) a secondary increase on day 5, and (3) subsequent sustained decreases on days 10 and 30 of estrogen replacement. This LH profile was paralleled by a similar pattern for α -subunit but not other glycoprotein hormones such as TSH or FSH. Rather, TSH values were invariant over time, while serum FSH concentrations declined continuously after estrogen administration. At the same time, serum prolactin concentrations increased significantly (days 1, 5, and 10 of estrogen), and then returned to baseline values by day 30. Accordingly, we infer that distinct and separable estrogen-sensitive mechanisms regulate mean serum concentrations of LH, FSH, and prolactin in postmenopausal women. Such estrogen-sensitive mechanisms could control patterns of hormone secretion as well as metabolic clearance.

After 5 days of estrogen replacement, the increase in serum LH concentrations was correlated significantly with a decrease in plasma free testosterone levels. This observation suggests that the increase in LH on day 5 may reflect not only estrogen's "positive" feedback effects on LH release but also a reduction in the negative feedback action of (unbound) testosterone on LH secretion. Further studies will be required to test the latter hypothesis.

Immunoactive growth hormone and somatomedin C concentrations did not change significantly in response to estradiol replacement. This observation is in accord with some previous studies and at variance with others, which suggest either increased or decreased growth hormone and somatomedin C concentrations in response to estrogen.¹⁶ Such disparities may reflect differences in estrogen dose or sampling or assay methodologies.

We conclude that the present model of selective es-

tradiol replacement in postmenopausal individuals should provide a means to investigate the distinct and separable estrogen-sensitive mechanisms that regulate the release of discrete anterior pituitary hormones in the human.

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Pharmacokinetics of nalbuphine during parturition

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Nalbuphine hydrochloride (Nubain) is a relatively new agonist-antagonist analgesic, chemically related to both oxymorphone and naloxone, which can be used as a systemic obstetric analgesic. After intravenous administration to six patients in labor at term, serial maternal serum specimens were obtained and assayed for their nalbuphine concentration. The maternal disposition of nalbuphine followed a two-compartment pharmacokinetic model, with an initial distribution phase of 4 to 20 minutes and a terminal elimination half-life of 2.4 ± 0.4 hours. Measurement of umbilical cord blood nalbuphine concentrations demonstrated that nalbuphine crossed the placenta and entered the fetal circulation. Newborn concentrations varied substantially, ranging from one third to six times the simultaneous maternal concentration. (AM J OBSTET GYNECOL 1986;155:340-4.)

Key words: Nalbuphine, pregnancy, pharmacokinetics, analgesia

Despite the widespread use of psychoprophylactic methods and epidural analgesia, systemic analgesics remain an integral part of intrapartum pain relief in modern-day obstetrics. Although many parenteral analgesics are available, meperidine is the best studied and probably the most frequently used systemic obstetric analgesic.¹

The concept of a single systemic narcotic-like medication providing analgesia and having narcotic antagonist characteristics was first realized with the introduction of pentazocine. This medication never achieved widespread use as an obstetric analgesic. Nalbuphine hydrochloride (Nubain) is another systemic narcotic agonist-antagonist. It is chemically related to both the opioid analgesic oxymorphone and the narcotic antagonist naloxone (Fig. 1). Human clinical studies indicate that its analgesic potency and duration of action are similar to those of morphine,² are three times as potent as pentazocine, and have a longer duration of action, yet it has fewer adverse hemodynamic effects and causes less nausea and vomiting.³ Although nalbuphine is approved and used as an obstetric analgesic, its pharmacokinetics have not been evaluated in pregnant women.⁴

The objectives of this study were to determine the

disposition of nalbuphine in pregnant women during labor and to quantitate newborn plasma nalbuphine concentrations at birth.

Material and methods

A total of 14 pregnant women between the ages of 18 and 32 years who requested systemic analgesia during active labor were initially entered into the study. All had had an unremarkable antepartum course and all were anticipated to have an uncomplicated vaginal delivery at the time of nalbuphine administration. Patients with a history or clinical evidence of renal or hepatic insufficiency, congestive heart failure, myocardial ischemia, or respiratory insufficiency were excluded from this study. Those patients who received an opioid drug within 12 hours of the administration of nalbuphine were also excluded from this study. In no case was there a history of drug abuse.

Maternal vital signs were obtained routinely during labor. Continuous electronic fetal heart rate recordings were kept on all patients throughout labor. The present study was approved by the Institutional Review Board and informed written consent was obtained from each patient.

Nalbuphine (15 to 20 mg) was administered as an intravenous bolus injection to parturients in active labor. To minimize the amount of placental transfer, the injection was given as close to the onset of a uterine contraction as possible. Maternal blood samples (4 ml) were scheduled for collection before (baseline) and at 3, 12, 20, 60, 120, 180, and 240 minutes or longer after nalbuphine administration, from an indwelling cannula located in a peripheral arm vein. In an effort to extend the data collection period and more accurately characterize the decay curve, the final blood specimen was

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Table I. Maternal and neonatal characteristics (mean $\pm$ SEM) of the 14 patients in the study group

Classification	Characteristic
Maternal	
Age (yr)	29.0 $\pm$ 2.2
Gravidity	2.2 $\pm$ 0.3
Weight (kg)	77.9 $\pm$ 4.6
Blood urea nitrogen (mg/dl)	6.6 $\pm$ 1.2
Creatinine (mg/dl)	0.7 $\pm$ 0.4
Neonate	
Gestational age (wk)	40.3 $\pm$ 0.7
Birth weight (gm)	3694.0 $\pm$ 343
Apgar score	
1 min	9.0 $\pm$ 0.0
5 min	9.8 $\pm$ 0.2

obtained as close to the time of delivery as possible. However, once the patient entered the delivery suite, no further maternal blood specimens were obtained. Umbilical artery samples were collected within 1 minute of the time of delivery. Specimens were refrigerated and allowed to clot. The plasma was then removed after centrifugation, immediately frozen, and stored at -25°C until assayed.

Isolation of nalbuphine was accomplished with the use of high-performance liquid chromatography with electrochemical detection. A one-step extraction technique was used. The following were respectively added to and then mixed with a 1 ml plasma sample: 50 ng of aqueous N-cyclopentyl methyl analogue of nalbuphine (internal standard), 250 μl of carbonate buffer at a pH of 10.0, and 2.0 ml of hexane:isoamyl alcohol in a 9:1 ratio. After centrifugation, the upper organic layer was removed, evaporated to a residue, and reconstituted in 75 μl of deionized water. Measurement of nalbuphine was performed by means of high-performance liquid chromatography on a C 18 column. The mobile phase for the liquid chromatography consisted of a mixture of methanol and phosphate buffer in a ratio of 55:65. The column eluate was monitored by a Perkin-Elmer (Model LC4BO) electrochemical amperometric detector with the use of a glassy carbon electrode. The oxidation of nalbuphine was accomplished at 0.75 V. Recording of the signal from the electrochemical detector was handled by a Hewlett-Packard (Model 3390A) recording integrator. The coefficient of variation of plasma nalbuphine is $<5\%$ at a nalbuphine concentration of 1 ng/ml.

The nalbuphine plasma concentrations for each patient were fit to the following biexponential equation:

$$C_p = Ae^{\alpha t} + Be^{\beta t}$$

where A is the zero-time intercept, α is the rate constant of the alpha distribution phase, B is the zero-time in-

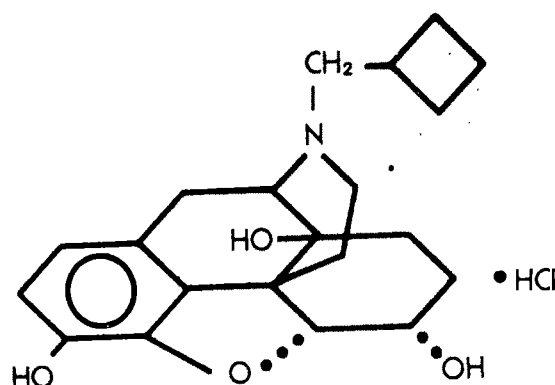


Fig. 1. Chemical structure of nalbuphine hydrochloride (Nubain).

tercept, and β is the rate constant of the beta elimination phase with the nonlinear least-squares regression computer program MULTI.⁵ Preliminary estimates used in the nonlinear data fits were generated by the method of residuals.⁶ The goodness of fit of the data points to their respective biexponential equations was assessed with the use of AIC (Akaike's information criterion) and coefficients of determination.^{7,8}

Pharmacokinetic variables for each pregnant woman were obtained by applying standard linear compartmental equations to the coefficients and exponents of the fitted curves. The uncertainty of the progression of labor, the limitation imposed by the investigators on the number of blood samples to be drawn, and the need to obtain maternal data points well distributed throughout the data collection period precluded the collection of a maternal blood specimen at birth. However, the plasma nalbuphine concentration of each fetal cord blood specimen was compared with its respective extrapolated maternal concentration by applying the biexponential equation of the maternal decay curve. All values are reported as the mean and SEM.

Results

The maternal analgesic response to nalbuphine was judged to be satisfactory in all cases. Side effects were limited to slight maternal drowsiness. There were no clinically significant changes in maternal vital signs. Fetal heart rate and long-term variability remained essentially unaltered both during and after the administration of nalbuphine. All newborn infants were discharged with their mothers after an uneventful course in the newborn nursery. Maternal and neonatal characteristics of all patients are listed in Table I.

Of the 14 patients entered into the study, seven were excluded from pharmacokinetic data analysis either because they were delivered of their infants before a complete set of maternal blood samples could be obtained

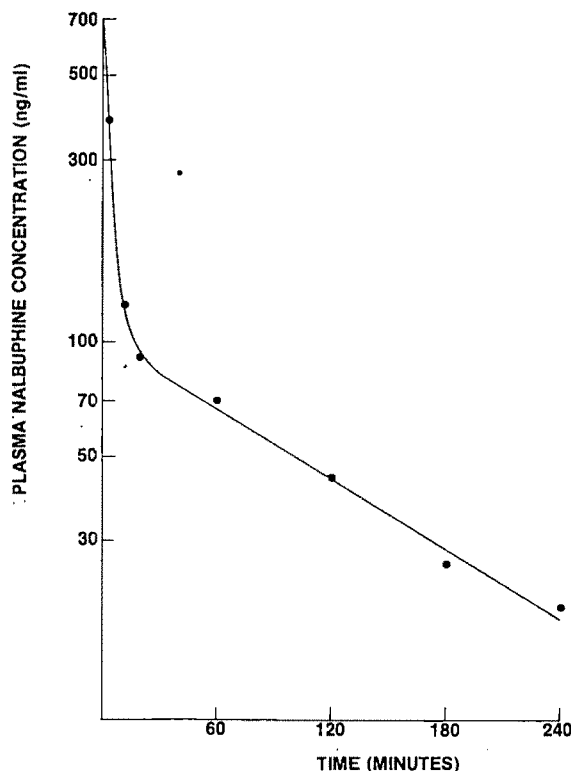


Fig. 2. Representative biexponential decay curve ($r^2 = 0.999$) of nalbuphine after a 20 mg intravenous bolus administration to a parturient patient (No. 76).

(four), because they required additional systemic analgesia before delivery (two), or because they had a cesarean delivery (one). Sufficient samples were obtained from the remaining seven patients; however, because of a laboratory accident, pharmacokinetic evaluations could be performed on only six patients.

Nalbuphine disposition after intravenous administration to the patient in labor followed a two-compartment pharmacokinetic model with a mean goodness of fit (r^2) of 0.997 (range 0.986 to 0.999). A representative curve fit to one of the patients is illustrated in Fig. 2. Table II lists the pharmacokinetic variables calculated for each patient. The initial distribution phase was essentially complete in 4 to 20 minutes. The half-life of the terminal elimination phase was 2.4 ± 0.4 hours. Systemic clearance ranged from 7.09 to 18.46 ml/min/kg and the mean steady-state volume of distribution was approximately 2 L/kg.

Maternal nalbuphine concentrations from 1 to 3 minutes after a 20 mg intravenous bolus ranged from 197 to 459 ng/ml (297 ± 44.1 ng/ml). Umbilical cord nalbuphine concentrations obtained between 180 and 582 minutes after maternal administration ranged from 4.2 to 46 ng/ml. The ratio of the extrapolated maternal nalbuphine plasma concentrations to the simultaneous fetal concentrations varied from 1:0.37 to 1:6.03

(Table III). The ratio could not be determined in Patient No. 19 because the fetal concentration was below the sensitivity of the assay. However, the maternal:fetal ratio in this patient was 1:<1.

Comment

This study clearly demonstrated that nalbuphine crosses the placenta into the fetal circulation. The magnitude and variability observed in the ratios of maternal:fetal nalbuphine plasma concentrations at birth were much greater than expected. Although a lack of neonatal depression suggests fetal insensitivity to nalbuphine, high fetal nalbuphine concentrations may relate in part to greater accumulation because of slower elimination from the fetus and in part to methodologic errors. Because the maternal concentration was extrapolated to the time at which the fetal cord specimen was obtained, a slight shift in the maternal decay curve would influence this ratio. Because fetal concentrations of nalbuphine at delivery were substantially higher and varied more with respect to maternal concentrations than anticipated, further work is needed in this area. Therapeutic maternal drug levels have not as yet been determined.

The limitations in this study are those inherent in obtaining data where only a limited number of specimens could be drawn from each patient. The precise characterization of the terminal elimination phase was hampered by the relatively short period of time during which data could be collected. The number of specimens obtained did not permit fitting the data to a triexponential equation and elimination from the peripheral compartment was assumed to be insignificant.

Other than a half-life of about 5 hours in healthy subjects and 3 to 3½ hours after multiple intravenous doses in patients undergoing cardiac surgery, there presently are no published data on the pharmacokinetics of nalbuphine in healthy adults for comparison.⁴ We resist implicating labor as the sole or predominant factor in the observed variability in half-life between pregnant women in labor and healthy adults because so little is known about the healthy subjects from whom these data were obtained. However, our observed mean half-life of 2 hours and 35 minutes suggests that the duration of action during labor may be shorter than for other indications.

The volume of distribution that we calculated for our group of women in labor (2 L/kg) was less than that of morphine (3.2 L/kg) or meperidine (3.8 L/kg) in healthy volunteers. Similarly the plasma clearance of nalbuphine during labor, 7 to 15 ml/min/kg, was less than the clearance of morphine or meperidine (mean approximately 14.5 ml/min/kg).^{9, 10} However, the distribution and clearance of nalbuphine may have been significantly influenced by the physiologic changes of

Table II. Pharmacokinetic variables of nalbuphine after intravenous administration to six pregnant women in labor

Patient No.	V _c (ml/kg)	V _{ss} (ml/kg)	k ₁₂ (L/min)	k ₂₁ (L/min)	k ₁₀ (L/min)	α (L/min)	t _{1/2} (β) (min)	CL (ml/min/kg)	AUC (ng · hr/ml)
80	87.5	2543.1	0.851	0.030	0.095	0.974	233.4	8.34	455.5
19	181.1	1520.5	0.127	0.017	0.039	0.179	185.3	7.09	639.0
31	482.1	2179.1	0.217	0.062	0.024	0.298	138.6	11.64	325.8
76	447.5	2246.6	0.170	0.042	0.041	0.247	97.9	18.46	280.0
97	437.6	1506.9	0.123	0.050	0.034	0.198	80.6	14.86	259.6
65	821.2	2195.3	0.102	0.061	0.018	0.175	110.7	14.72	254.1
Mean	409.5	2031.9	0.265	0.044	0.042	0.345	141.1	12.52	369.0
SEM	105.3	172.5	0.118	0.007	0.011	0.129	23.8	1.76	62.0

V_c = Central volume of distribution; V_{ss} = steady-state volume of distribution; k₁₂ = rate of transfer from the central to the peripheral compartment; k₂₁ = rate of transfer from the peripheral to the central compartment; k₁₀ = rate of elimination from the central compartment; α = exponent of the biexponential curve: C_p = Ae^α + Be^β; t_{1/2}(β) = elimination half-life; CL = total body clearance; AUC = area under the curve (zero to infinity).

Table III. Simultaneous maternal and umbilical artery nalbuphine plasma concentrations after intravenous administration to six pregnant women in labor

Patient No.	Fetal C _p (ng/ml)	Time of fetal C _p (hr)	Extrapolated maternal C _p (ng/ml)	M:F	Maternal injection (mg)
80	10.0	5.58	27.2	1:0.37	20
19	—*	10.03	12.0	—*	20
31	46.0	3.00	37.1	1:1.24	15
76	11.0	6.52	6.4	1:1.72	20
97	4.2	7.38	2.6	1:1.63	20
65	14.0	9.70	2.3	1:6.03	20

C_p = Plasma concentration; M:F = ratio of the extrapolated maternal nalbuphine plasma concentration to the simultaneous umbilical artery concentration.

*Below the limit of sensitivity of the assay.

labor. During pregnancy an increased maternal plasma volume, altered plasma protein concentrations, and increased cardiac output predispose to altered drug distribution.¹⁻¹² It was therefore not unexpected that the steady-state volume of distribution and the plasma clearance of meperidine were shown to be significantly lower in women during labor when compared with nonpregnant control subjects.¹³ Until data on nalbuphine in nonpregnant subjects become available, the significance of labor in these two important dosing parameters is uncertain. Nevertheless, the short distribution phase and relatively large volume of distribution of nalbuphine suggest that there is substantial distribution to highly perfused tissues during labor.

Two nalbuphine metabolites in addition to its conjugates have been identified in the urine of human subjects. Their pharmacologic properties, if any, have yet to be identified. The percentage of a nalbuphine dose that undergoes hepatic metabolism and the extent to which it is extracted by the liver have not been reported.⁴ However, comparative efficacy data with oral and parenteral administration suggest substantial first-pass hepatic metabolism.¹⁴ This being the case, nalbuphine is likely to have a high hepatic extraction ratio,

similar to that of morphine, pentazocine, and meperidine. This may explain in part the large interpatient variability observed for most of the pharmacokinetic variables in pregnant women (Table II) and suggests that nalbuphine distribution and clearance are closely related to the hemodynamic changes observed during labor.

The respiratory depression that can be seen after the administration of nalbuphine is similar at recommended doses to that produced by an equianalgesic dose of morphine and other narcotics. However, unlike opioids, nalbuphine has the safety advantage of having a respiratory depression ceiling effect beyond which larger or repeated doses do not cause a greater intensity of or increase the duration of respiratory depression.^{3,15} Because of this ceiling effect, it may be possible to give multiple doses or increase the amount of nalbuphine to provide greater degrees of analgesia without significantly increasing the risk of maternal respiratory depression.

The fetus is normally acidotic with respect to its mother. Thus the 8.7 pKa of nalbuphine, like that of the high pKa values of narcotics and their metabolites, will promote diffusion of the substance to the fetal side

of the placenta.² This ion-trapping effect permits an even greater fetal drug accumulation during episodes of fetal acidosis.¹⁶ However, because of the narcotic antagonist properties and respiratory ceiling effect of nalbuphine, equianalgesic doses may cause less neonatal depression than other narcotics.

In the population studied, nalbuphine provided adequate maternal analgesia without serious side effects. Although it was distributed into the fetal circulation to concentrations estimated to approximate or surpass maternal levels at delivery, there were no apparent fetal or newborn adverse effects. However, further assessment needs to be undertaken to determine if this medication or its metabolites have subtle neonatal neurobehavioral effects.

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Maternal ingestion of acetylsalicylic acid inhibits fetal and neonatal prostacyclin and thromboxane in humans

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Small doses of maternal acetylsalicylic acid have proved to prevent preeclampsia. To study the mechanism of this action of acetylsalicylic acid, healthy women ingested 100 mg ($n = 13$) or 500 mg ($n = 14$) of acetylsalicylic acid during labor at term. The fetal prostacyclin synthesis, as assessed by the production of 6-ketoprostaglandin $F_{1\alpha}$ (a metabolite of prostacyclin) by the umbilical artery, was reduced from 21.3 ± 1.6 ng/gm/min of dry weight in the controls ($n = 25$, mean $\pm$ SE) to 7.8 ± 1.1 ng/ml/min ($p < 0.001$) in infants of mothers receiving 500 mg of acetylsalicylic acid, but it was unchanged in infants with mothers receiving 100 mg of acetylsalicylic acid (19.5 ± 2.3 ng/gm/min). Maternal ingestion of 500 mg of acetylsalicylic acid also was accompanied by reduced ($p < 0.10$) urinary excretion of 6-ketoprostaglandin $F_{1\alpha}$ in neonates during the first 3 days of life. The fetal platelet thromboxane A_2 synthesis, as assessed by the release of thromboxane B_2 (a metabolite of thromboxane A_2) during spontaneous clotting of the umbilical blood (63.4 ± 4.2 pg/ 10^5 platelets, $n = 22$), was inhibited in infants born to mothers given 100 mg (14.0 ± 3.7 pg/ 10^5 platelets, $p < 0.001$) or 500 mg of acetylsalicylic acid (6.1 ± 3.5 pg/ 10^5 platelets, $p < 0.001$). The thromboxane B_2 release by the umbilical artery (1.1 ± 0.1 ng/gm/min, $n = 13$) also was decreased in infants of mothers receiving 500 mg of acetylsalicylic acid (0.57 ± 0.1 ng/gm/min, $n = 7$, $p < 0.01$). Thus a small dose of maternal acetylsalicylic acid (100 mg) inhibits only the fetoplacental thromboxane A_2 but leaves prostacyclin production unaffected. (AM J OBSTET GYNECOL 1986;155:345-9.)

Key words: Pregnancy, acetylsalicylic acid, prostacyclin, thromboxane

Two recent reports have suggested a potential usefulness of small daily doses of acetylsalicylic acid (aspirin) in the prevention of acute¹ and superimposed preeclampsia.² This effect was assumed to be mainly due to acetylsalicylic acid-induced inhibition in the platelet-activating and vasoconstricting thromboxane A_2 ,^{1,2} particularly, because there is a relative overproduction of thromboxane A_2 in comparison to the vasodilating prostacyclin in fetoplacental tissues in preeclampsia, as summarized recently.³ There are, however, no direct data to support this theory, which is further challenged by the fact that acetylsalicylic acid can also inhibit the synthesis of prostacyclin. Thus the hypothesis can hold only in the case that there is a proper dose of acetylsalicylic acid which inhibits thromboxane A_2 synthesis but leaves prostacyclin synthesis

unaffected in fetoplacental tissue. We have studied the concomitant effect of maternal ingestion of various doses of acetylsalicylic acid on the fetal and neonatal prostacyclin and thromboxane A_2 .

Material and methods

Subjects. Twenty-seven healthy parturients with term normotensive pregnancies and vaginal deliveries were studied with the approval of the committee of ethics of the University of Oulu after being thoroughly informed about the purpose and course of the investigation (Table I). Participants had not taken any drugs for at least 10 days preceding the investigation. Labor began spontaneously and during it the women ingested in randomized order 100 mg or 500 mg of acetylsalicylic acid with 100 ml of water 28 to 658 minutes before delivery (Table I). The uterine contractions and fetal heartbeat were recorded continuously before and after acetylsalicylic acid intake. Thirty-five comparable women not receiving acetylsalicylic acid were studied as controls. All study subjects gave birth spontaneously to healthy infants with 1-minute Apgar scores of ≥ 8 .

Measurement of fetal and neonatal prostacyclin. Fetal prostacyclin generation was measured from the specimens of umbilical arteries by use of a tissue su-

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Table I. Clinical data of various study groups (mean $\pm$ SE)

	Maternal acetylsalicylic acid		Controls
	100 mg	500 mg	
No. of women*	13	14	35
Age (yr)	28.5 $\pm$ 1.9	27.0 $\pm$ 1.0	28.1 $\pm$ 0.8
Range	17 – 37	20 – 33	19 – 34
Parity			
Nulliparous	4	6	12
Parous	9	8	23
Gestational age at delivery (wk)	40.2 $\pm$ 0.2	40.7 $\pm$ 0.2	39.8 $\pm$ 0.4
Range	39 – 42	39 – 42	38 – 42
Birth weight (gm)	3700 $\pm$ 152	3790 $\pm$ 149	3650 $\pm$ 79
Range	3020 – 4500	3080 – 5170	2940 – 417
Time from acetylsalicylic acid intake to birth (min)	344 $\pm$ 71	243 $\pm$ 36	
Range	35 – 658	28 – 515	
Concentration of salicylate in umbilical cord blood (μ mol/l)	34.0 $\pm$ 7.4	131.6 $\pm$ 32.4*	
Range	10 – 86	21 – 486	

*p < 0.0125 in comparison between acetylsalicylic acid groups.

Table II. Production of 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 by the umbilical artery, fetal platelets' capacity to synthesize thromboxane B_2 , and neonatal urinary excretion of 6-ketoprostaglandin $F_{1\alpha}$ in infants of mothers with acetylsalicylic acid treatment during labor (mean $\pm$ SE)

	Umbilical production (ng/gm/min)						Neonatal urinary 6-keto prostaglandin $F_{1\alpha}$ (ng/mmol creatinine)			
	6-keto prostaglandin $F_{1\alpha}$		$Tx B_2$				1st day		3rd day	
	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE
Control group	25	21.3 $\pm$ 1.6	25	1.10 $\pm$ 0.1	22	63.4 $\pm$ 4.2	13	317.7 $\pm$ 43.7	10	133.3 $\pm$ 17.7
Maternal ASA 100 mg	13	19.5 $\pm$ 2.3	13	0.83 $\pm$ 0.08	11	14.0 $\pm$ 3.7*	6	309.2 $\pm$ 31.2	5	114.5 $\pm$ 18.7
Maternal ASA 500 mg	14	7.8 $\pm$ 1.1*‡	7	0.57 $\pm$ 0.1†	11	6.1 $\pm$ 3.5*	13	204.3 $\pm$ 25.1§	10	79.9 $\pm$ 13.3§

*p < 0.001 in comparing to the control group.

†p < 0.01 in comparing to the control group.

‡p < 0.01 in comparing between acetylsalicylic acid group.

§p < 0.10 in comparing to the control group.

perfusion method.⁴ Briefly, umbilical artery samples (10 to 20 mg of dry weight) were placed into plastic chambers and superfused with Eagle's medium (pH 7.4; 37° C; carbon dioxide/oxygen, 5%:95%). After a wash-out period of 2.5 hours, a 1-hour fraction was collected and its content of 6-ketoprostaglandin $F_{1\alpha}$ (a stable hydration product of prostacyclin) was measured by radioimmunoassay.⁵ Results are expressed as nanograms of 6-ketoprostaglandin $F_{1\alpha}$ produced per minute per gram of dry weight tissue. The storage of the sample in the frozen state did not affect its capacity to produce prostacyclin.⁴ The recovery of 6-ketoprostaglandin $F_{1\alpha}$ added to perfusion buffer was 95.3% $\pm$ 3.3% (n = 10, mean $\pm$ SD).

Neonatal prostacyclin was studied by measuring the concentration of 6-ketoprostaglandin $F_{1\alpha}$ in urine. Urine samples were collected into plastic bags on the first and third day of life. Urine samples (2 ml) were acidified to pH 3.0 with 1N hydrochloric acid and passed through an octadecylsilyl silica cartridge (Sep-

Pak C₁₈ cartridges, Waters Chromatography Division of Milipore, Milford, Massachusetts), prewashed successively with 5 ml of ethanol and 5 ml of petroleum ether. The 6-ketoprostaglandin $F_{1\alpha}$ absorbed was eluted in 2 ml of ethylacetate, which was evaporated to dryness under a nitrogen stream. The sample was further purified with high performance liquid chromatography (Spectron Physics M-740, Santa Clara, California) with use of a Sherisorb 5 ODS II column (Phase Separations Ltd., Industrial Estate Queensberry, CLWD, United Kingdom) and a mobile phase of water/acetonitrile/acetic acid (69.95:30.00:0.05) with flow rate of 2 ml/min. The fraction containing 6-ketoprostaglandin $F_{1\alpha}$ (5 to 6 ml) was collected, evaporated to dryness, and dissolved in phosphate-buffered saline solution with 0.1% of gelatin. Two different-size aliquots, both in duplicate, were taken for radioimmunoassay.⁵ The recovery of 6-ketoprostaglandin $F_{1\alpha}$ added into urine (200 to 400 pg/ml) was 79.6% $\pm$ 8.9% (mean $\pm$ SD, n = 10) and the interassay variation 10.8% (n = 10) after the

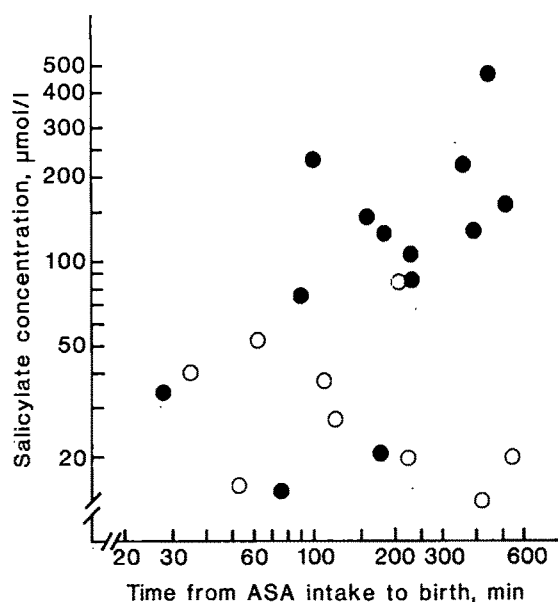


Fig. 1. Salicylate concentrations in umbilical sera of infants born to mothers given 100 mg (○) or 500 mg (●) of acetylsalicylic acid (ASA) during labor.

entire procedure. To eliminate the effect of changes of the water content of urine, the results are given as nanograms per millimole of creatinine determined by a routine laboratory method.

Measurement of fetal thromboxane. The vascular production of thromboxane A_2 was measured by assaying the concentrations of thromboxane B_2 (a metabolite of thromboxane A_2) from the superfusate of the umbilical artery.⁵ To study the fetal platelets' capacity to produce thromboxane A_2 , umbilical vein blood samples were collected into dry plastic tubes and allowed to clot spontaneously at 37° C for 60 minutes. Serum was separated, and its concentration of thromboxane B_2 was measured by radioimmunoassay.⁶ The results are expressed as picograms of thromboxane B_2 /10⁵ platelets.

Measurement of salicylate concentration. Serum samples from the mixed umbilical cord blood were collected for the salicylate measurement. To this end, ferric nitrate was added to serum, and the salicylate concentration calculated from the light absorbance of the end product at 540 nm. When measured by this method, the therapeutic serum salicylate concentration was between 500 to 2500 μmol/L.

Statistical analysis. The *t* test modified according to the Bonferroni method⁷ and linear regression analysis were employed for the statistical analysis of the results.

Results

Maternal and fetal findings. The study groups were comparable in regard to the maternal, fetal, and neonatal findings (Table I). Doses of acetylsalicylic acid did not change uterine contractility or fetal heart rate. The

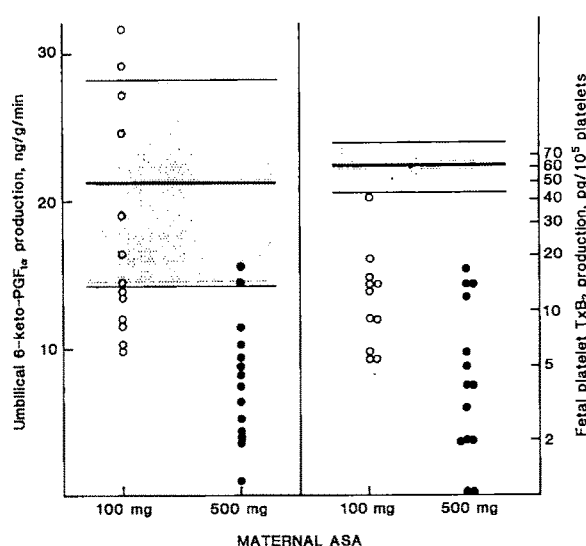


Fig. 2. Production of 6-ketoprostaglandin $F_{1\alpha}$ by the umbilical artery and release of thromboxane B_2 (TxB_2) by the platelets in infants born to the mothers given 100 mg (○) or 500 mg (●) of acetylsalicylic acid (ASA) during labor. The shaded area indicates the normal mean $\pm$ 1 SD.

blood-loss during delivery was <500 ml in each subject. No clinically detectable bleeding tendency or any other adverse effects were seen in newborn infants of mothers given acetylsalicylic acid during labor.

Salicylate level. The interval from acetylsalicylic acid intake to birth did not differ significantly between the two acetylsalicylic acid-treated groups (Table I). The salicylate was detectable in umbilical blood in each infant, and its concentration was higher after the 500 mg dose than the 100 mg dose of maternal acetylsalicylic acid (Table I and Fig. 1). The salicylate level was close to the adult therapeutic range only in one newborn infant (486 μmol/L) whose mother received 500 mg of acetylsalicylic acid 455 minutes before delivery.

Fetal and neonatal prostacyclin. Maternal ingestion of 100 mg of acetylsalicylic acid had no effect on the umbilical 6-ketoprostaglandin $F_{1\alpha}$ production (Table II and Fig. 2) or on the neonatal urinary 6-ketoprostaglandin $F_{1\alpha}$ excretion (Table II), whereas the 500 mg dose reduced both fetal and neonatal prostacyclin production (Table II and Fig. 2). This inhibition was not related to the interval between acetylsalicylic acid intake and delivery or to the salicylate level in the umbilical blood.

Fetal and neonatal thromboxane. Maternal administration of 100 mg of acetylsalicylic acid inhibited the thromboxane A_2 synthesis in fetal platelets (Table II and Fig. 2) but not in umbilical artery, whereas the 500 mg dosage was accompanied by reduced thromboxane A_2 synthesis by both of them. The acetylsalicylic acid-induced inhibitions in the platelets' thromboxane A_2 synthesis were related to the salicylate levels in umbilical blood (Fig. 3), although no statistically significant dif-

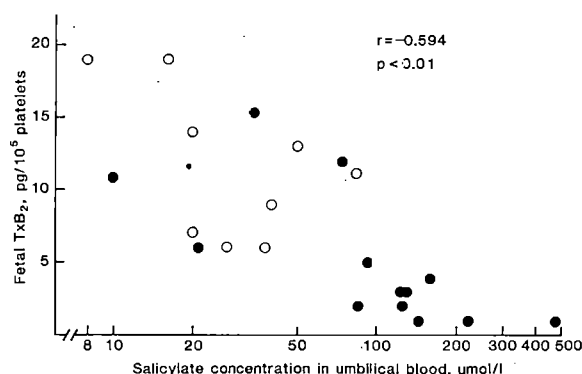


Fig. 3. Release of thromboxane B_2 (TxB_2) by the fetal platelets in relation to the salicylate concentration in the umbilical blood in infants born to mothers given 100 mg (○) or 500 mg (●) of acetylsalicylic acid during labor.

ference in the mean thromboxane B_2 releases per 10^5 platelets was seen between the acetylsalicylic acid groups.

Comment

We report the concomitant effects of small doses of maternal acetylsalicylic acid both on fetal and neonatal prostacyclin/thromboxane A_2 production. Maternal acetylsalicylic acid was transported to the fetus, as shown by the presence of salicylate in fetal circulation and by the inhibition of fetal prostanoid synthesis. Acetylsalicylic acid inhibits thromboxane A_2 synthesis in adult platelets by irreversibly acetylating the platelet cyclooxygenase.⁸ Our data show that 100 mg and 500 mg of acetylsalicylic acid given to mothers also inhibit the fetal platelet thromboxane A_2 synthesis in relation to the salicylate level in umbilical blood. The reduction of fetal platelet thromboxane A_2 synthesis in the present work was, however, much less than what occurred in adult platelets after a single dose of acetylsalicylic acid.⁹ This does not, however, necessarily indicate a difference in the sensitivity of fetal and adult platelets to the inhibitory action of acetylsalicylic acid. The explanation is probably pharmacokinetic. The half-life of acetylsalicylic acid in blood is only 12 to 20 minutes, and a great deal of orally ingested acetylsalicylic acid is quickly hydrolyzed to salicylate during absorption in the gastrointestinal tract.^{10,11} Thus it is conceivable that only a minor fraction of maternal acetylsalicylic acid, if any, enters the fetal circulation as an intact compound. The main metabolite of acetylsalicylic acid, salicylate, is a much weaker cyclooxygenase inhibitor than acetylsalicylic acid itself. This may explain why fetal platelet thromboxane A_2 production was only partially inhibited even after a 500 mg dose of maternal acetylsalicylic acid. The fetal vascular thromboxane A_2 synthesis was even more resistant to the effect of maternal acetylsal-

icylic acid than was fetal platelet thromboxane A_2 synthesis, which is compatible with the *ex vivo* findings on the difference between the sensitivity of fetal platelet and vascular cyclooxygenases against cyclooxygenase inhibitors.¹²

Maternal acetylsalicylic acid ingestion as a single dose inhibited the fetal and neonatal prostacyclin synthesis. Interestingly, this effect was seen only with the 500 mg but not the 100 mg dose. Thus our data suggest that a small dose of maternal acetylsalicylic acid can direct the fetoplacental prostacyclin/thromboxane A_2 balance to the dominance of prostacyclin, because it inhibits thromboxane A_2 synthesis more readily than prostacyclin. Thus our present findings may reveal the biochemical mechanism by which 60 mg or 150 mg of acetylsalicylic acid daily prevented acute¹ and superimposed² preeclampsia, respectively. Keeping in mind the irreversible effect of acetylsalicylic acid on the platelet thromboxane A_2 synthesis⁸ and a platelet life-span about 10 days, these doses of acetylsalicylic acid could perhaps have been given only at 5 to 7 days intervals. In this respect the rationale would be much the same as in trials conducted to prevent occlusive arterial disease with acetylsalicylic acid.^{13,14} Nevertheless, caution is needed. Although we and the others^{1,2,15} did not encounter neonatal bleeding or other side effects, such possibility may exist¹⁶ even with administration of small doses of acetylsalicylic acid, because in this circumstance, bleeding-promoting prostacyclin dominates in the fetus.

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Potassium regulation and progesterone-aldosterone interrelationships in human pregnancy: A prospective study

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Little is known about the intrinsic renal and hormonal regulation of potassium excretion in pregnancy despite major alterations in many of the potassium regulatory factors. Forty primigravid women on an unrestricted diet were studied during the second and third trimesters and exhibited constant absolute and fractional potassium excretion despite a significant increase in plasma aldosterone concentration between these stages. The plasma progesterone level rose significantly between studies but closer analysis showed no correlation between individual changes in plasma aldosterone concentration and progesterone between trimesters. In the 14 subjects studied post partum, baseline absolute potassium excretion was not significantly altered but filtered potassium fell and fractional potassium excretion tended to rise. After dietary sodium manipulation at these stages, absolute potassium excretion, fractional potassium excretion, and progesterone were unaltered despite significant changes in plasma aldosterone concentration and sodium excretion. These results suggest that potassium excretion is held constant throughout pregnancy and that renal tubular potassium reabsorption adjusts appropriately to the increased filtered potassium load. Progesterone does not appear to be involved in the acute regulation of potassium or sodium excretion but may have effects on sodium and potassium excretion that are constant, proportional to its placental production, and unresponsive to endogenous changes in mineralocorticoid production. (*Am J Obstet Gynecol* 1986;155:349-53.)

Key words: Progesterone, aldosterone, potassium, sodium

It is surprising that the normal pregnant woman is not potassium depleted. Not only is there a 50% increase in glomerular filtration rate and therefore in filtered sodium and potassium, but urine flow rate, as well as aldosterone, cortisol, and deoxycorticosterone acetate¹ production are increased from the nonpreg-

nant state. In addition, plasma arginine vasopressin content appears to be increased² and there is a mild alkalosis. All of these factors are capable of promoting urinary potassium loss and hypokalemia either independently or in concert, and mineralocorticoid production increases further as pregnancy progresses, aggravating this potassium-losing tendency. However, there is an overall calculated cumulative retention of approximately 350 mmol of potassium throughout normal pregnancy.³

The mechanisms responsible for maintaining this net positive accumulation of potassium are unknown although an antidiuretic role has been ascribed to progesterone,⁴ whose production increases throughout pregnancy and parallels that of aldosterone.⁵ However,

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Table I. Baseline potassium and sodium excretion, glomerular filtration rate, and plasma aldosterone and progesterone concentrations during and after pregnancy

	<i>n</i>	<i>Absolute potassium excretion (mmol/day)</i>	<i>Plasma potassium (mmol/L)</i>	<i>Glomerular filtration rate (ml/min/1.73 m²)</i>	<i>Fractional potassium excretion (%)</i>	<i>Absolute sodium excretion (mmol/day)</i>	<i>Plasma aldosterone concentration (fmol/ml)</i>	<i>Progesterone (nmol/L)</i>
Second trimester	40	65 ± 4	3.7 ± 0.03	152 ± 5	8.0 ± 0.4	109 ± 7	1263 ± 116	251 ± 16
Third trimester	40	64 ± 4	3.7 ± 0.04	148 ± 5	8.3 ± 0.5	103 ± 6	2170 ± 162*	540 ± 69†
Post partum	14	58 ± 7	4.1 ± 0.10‡	99 ± 4‡	10.3 ± 1.1	100 ± 10	500 ± 126‡	—

**p* < 0.001, between trimesters.†*p* < 0.01, between trimesters.‡*p* < 0.0001, third trimester versus postpartum.

the interrelationship between aldosterone and progesterone remains controversial because of the finding that plasma levels of these hormones do not always correlate significantly during pregnancy⁶ and the use of exogenous progesterone in many experiments, which may not mimic physiologic events. Overall, the balance of evidence to date suggests that progesterone is capable of offsetting the potassium-excreting influence of aldosterone, although it is not clear to what extent, if any, this hormone is involved in the day-to-day regulation of potassium homeostasis. If such an effect does occur during pregnancy, then it might reasonably be expected that there is a positive correlation between endogenous production of progesterone and aldosterone, as evidenced by their plasma concentrations.

This study was performed to examine the short- and long-term interrelationships between aldosterone, progesterone, and potassium excretion in normal pregnancy. This involved measurement of baseline absolute and fractional potassium and sodium excretion in the context of filtered potassium and plasma aldosterone and progesterone concentrations in the second and third trimesters and post partum. In addition to baseline studies, endogenous aldosterone production was manipulated by altering sodium intake at these same stages, and subsequent absolute and fractional urine potassium excretion was examined in the light of plasma progesterone concentrations.

Material and methods

Study protocol. Forty primigravid women, all outpatients of the Royal North Shore Hospital, gave written informed consent and were studied during both the second (22 to 26 weeks) and third (32 to 36 weeks) trimesters. Fourteen of these subjects had the same studies again between 3 and 9 months post partum.

All subjects had studies made under baseline conditions of unrestricted eating at each stage. Urine was

collected for 48 hours for measurement of sodium, potassium, creatinine, and volume. The next morning (day 0), blood was collected between 7:00 and 10:00 AM for plasma sodium, potassium, true endogenous creatinine, aldosterone, and progesterone concentrations.

All were then allocated randomly to either a high-salt diet group or a low-salt diet group and each followed this same diet for 1 week during each study. The high-salt diet consisted of a dietary intake of approximately 150 mmol/day, supplemented with sodium chloride capsules (17 mmol of ionized sodium per capsule) to a total intake of between 250 and 300 mmol of sodium per day. Those allocated to the low-salt group were instructed in a diet of approximately 20 mmol per day. Potassium intake was not strictly controlled but was not expected to be significantly altered by the institution of these diets. Urine was collected for the last 48 hours of the diet and blood was collected at the completion of the diet (day 7), at the same time of day, for measurement of the same parameters that were determined in the baseline state. The plasma progesterone level was not measured in the postpartum studies.

Analytical methods. From the parameters determined above, measurements were made of 24-hour urinary sodium and potassium excretion, glomerular filtration rate as the endogenous creatinine clearance, and the fractional excretion of potassium. The fractional excretion is the percentage of filtered potassium appearing in the final urine specimen.

Urinary sodium and potassium levels were measured by flame photometry (Instrument Laboratories, Sydney) and creatinine was determined spectrophotometrically after reaction with alkaline picrate. Plasma sodium and potassium levels were determined by an AutoAnalyzer method and plasma true endogenous creatinine was determined spectrophotometrically after extraction and adsorption to Fuller's earth. Plasma

Table II. Potassium and sodium excretion in response to dietary sodium manipulation during and after pregnancy

	Day	High-salt diet			Low-salt diet		
		Second trimester	Third trimester	Post partum	Second trimester	Third trimester	Post partum
n		20	20	7	20	20	7
Absolute sodium excretion (mmol/day)	0	99 ± 7	98 ± 8	90 ± 16	118 ± 12	105 ± 9	110 ± 12
	7	274 ± 13*	256 ± 18*	259 ± 27*	27 ± 3*	26 ± 3*	13 ± 4*
Absolute potassium excretion (mmol/day)	0	59 ± 5	59 ± 6	59 ± 12	67 ± 6	61 ± 5	58 ± 7
	7	63 ± 5	64 ± 6	66 ± 12	64 ± 3	58 ± 4	55 ± 7
Fractional potassium excretion (%)	0	7.9 ± 0.5	9.0 ± 1.0	10.2 ± 1.2	8.0 ± 0.6	7.4 ± 0.7	10.1 ± 1.2
	7	8.3 ± 0.7	8.5 ± 0.9	10.3 ± 1.7	8.6 ± 0.4	8.0 ± 0.6	10.5 ± 1.6

* $p < 0.001$, day 0 versus day 7.

progesterone (Amerlex-M progesterone radioimmunoassay kit, Amersham, United Kingdom) and aldosterone⁷ levels were measured by radioimmunoassay.

Statistics. Differences between means within groups were sought by paired Student's *t* test and those between groups by the unpaired Student's *t* test. Correlations were determined by calculation of the correlation coefficient (*r*) and their significance was determined from the analysis of variance. Results are expressed as mean ± SEM.

Results

Baseline studies (Table I). There was no significant difference in absolute or fractional potassium excretion, plasma potassium, or glomerular filtration rate between trimesters. Plasma potassium was higher and glomerular filtration rate significantly lower post partum, leading to a reduction in filtered potassium at this stage. Urinary potassium was not significantly altered and, although fractional potassium excretion was higher, the difference did not achieve statistical significance. When these data were analyzed for only 14 subjects studied at each stage, the results were almost identical. Plasma progesterone concentration and plasma aldosterone concentration were elevated in pregnancy and rose significantly between trimesters.

There was a significant overall correlation between plasma aldosterone concentration and progesterone ($r = 0.39$, $p < 0.001$), which was apparent during the second trimester ($r = 0.44$, $p < 0.01$) but not the third ($r = 0.17$, NS). Furthermore, no correlation was apparent between the change in plasma aldosterone concentration and the change in progesterone between trimesters for individual subjects ($r = 0.06$, NS).

Dietary sodium manipulations. Urinary sodium excretion rose significantly ($p < 0.001$) after the high-salt diet and fell after the low-salt diet ($p < 0.001$) but potassium excretion and fractional potassium excretion

remained constant at all stages (Table II). During pregnancy, the plasma aldosterone concentration fell after sodium loading and rose significantly after sodium deprivation, but plasma progesterone was not significantly altered during these studies (Table III). Similar patterns were observed for plasma aldosterone concentration post partum.

Comment

These results demonstrate that baseline absolute urinary potassium excretion on an unrestricted diet is similar during pregnancy to that in the nonpregnant state but suggest that renal tubular handling of potassium is altered during pregnancy. Furthermore, the close regulation of potassium excretion during pregnancy is maintained during the second and third trimesters despite marked alterations in circulating levels of aldosterone. Finally potassium excretion is unaltered by large variations in salt intake at these stages of pregnancy, despite further alterations in endogenous mineralocorticoid production and a constant plasma progesterone concentration.

The factors regulating renal excretion of potassium are complicated although it is generally held that the distal segments of the nephron determine final urinary potassium excretion. Recently, Field et al.,⁸ in a series of carefully designed experiments, assessed the independent effects of arginine vasopressin, aldosterone, and urine flow rate on potassium excretion. They showed that potassium excretion is promoted by arginine vasopressin and aldosterone and is directly related to urine flow rate and suggested that overall homeostasis is maintained by a constant interplay of these factors.

Our data confirm that, under basal conditions of unrestricted eating, net urinary potassium excretion alters little during pregnancy, but there appears to be some differences in the mechanisms regulating potassium

Table III. Plasma aldosterone and progesterone concentrations in response to dietary sodium manipulations during and after pregnancy

	Day	High-salt diet			Low-salt diet		
		Second trimester	Third trimester	Post partum	Second trimester	Third trimester	Post partum
n		20	20	7	20	20	7
Plasma aldosterone concentration (fmol/ml)	0	1127 ± 156	2156 ± 241	684 ± 231	1333 ± 153	2472 ± 321	502 ± 166
	7	840 ± 105*	1152 ± 117†	163 ± 26‡	2694 ± 328†	3846 ± 319†	871 ± 226*
Progesterone (nmol/L)	0	247 ± 16	667 ± 82	—	242 ± 17	419 ± 34	—
	7	248 ± 18	673 ± 90	—	267 ± 22	484 ± 30	—

*p < 0.05, day 0 versus day 7.

†p < 0.001, day 0 versus day 7.

‡p < 0.01, day 0 versus day 7.

homeostasis at these stages. Assessment of both filtered potassium and fractional potassium excretion are dependent on accurate measurement of the glomerular filtration rate, but there is little doubt that true glomerular filtration rate is difficult to determine, particularly during pregnancy. The most commonly used method for determining glomerular filtration rate, the use of 24-hour creatinine clearance, was the method employed in this study. This method has been criticized as it can be influenced by incomplete urine collections and false estimation of plasma creatinine concentrations with AutoAnalyzer techniques. To minimize these errors, we extended the duration of urine collection and measured true (endogenous) creatinine after extraction and absorption procedures. Completeness of collection was confirmed in each case by comparison with calculated expected values for age and sex. Other workers,⁹ in carefully executed studies, have demonstrated a very good correlation between inulin and creatinine clearances in pregnancy and, although the absolute values did not always agree, concluded that 24-hour creatinine clearance is a useful method of assessing glomerular filtration rate in pregnancy.

There was a significant reduction in baseline glomerular filtration rate and, despite the rise in plasma potassium, an overall reduction in filtered potassium in our subjects when studied post partum. This was accompanied by a rise in fractional potassium excretion from pregnant values. The calculated postpartum reduction in filtered potassium of 26% appears to have been "balanced" by the 24% increase in the fraction of this filtered potassium load that appeared in the final urine, that is, the fractional potassium excretion. An alternative view of these data is that, during pregnancy, filtered potassium increased by 35% but its fractional excretion decreased by only 19%, leading to net potassium loss. This concept could still be in keeping with an overall accumulation of <2 mmol of potassium per day in pregnancy and would be understandable in view

of the high circulating levels of mineralocorticoids, but only serial metabolic studies of potassium regulation on a fixed potassium intake can resolve whether this view is correct.

Nevertheless it is clear that although the plasma potassium level does fall during pregnancy, the normal pregnant woman does not develop frank hypokalemia in the face of the markedly increased filtered potassium load and mineralocorticoid activity. Whatever mediates this renal tubular adaptation is unknown but it has been proposed that progesterone might be a major antidiuretic factor that is operative during pregnancy.⁴ In light of this proposal, the demonstrated rise in plasma progesterone concentration between the second and third trimesters in our subjects was appropriate.

The evidence for both antimineralocorticoid and antidiuretic actions of progesterone remain controversial. In some studies, parallel changes have been observed between progesterone and aldosterone excretion¹⁰ and concentration,⁵ but others have found no such correlation.⁶ Evidence has been accumulated attesting to the mineralocorticoid-opposing effects of exogenous, usually intramuscular, progesterone, but whether these effects are similar to the direct actions of endogenous progesterone is uncertain. More direct studies seem to substantiate an antimineralocorticoid action for progesterone,¹¹ but even in these experiments it was observed that deoxycorticosterone acetate, a product of progesterone, was also capable of causing significant displacement of aldosterone from its receptor sites, raising the possibility that the effect of exogenous progesterone on mineralocorticoids is partly due to deoxycorticosterone acetate.

The early observation that a subject with hyperaldosteronism was able to cease potassium supplements as progesterone production increased during pregnancy¹² was not substantiated in a recent similar report.¹³ Hence there is considerable confusion over the role of progesterone in the regulation of potassium

excretion in pregnancy and few data exist that address potassium balance in pregnancy overall.

Our results do not dispute the previously observed parallel increases in progesterone and aldosterone throughout pregnancy, and we observed a weak but significant correlation between unmanipulated levels of these hormones in this study. Closer examination of this relationship demonstrated that it was present only in the second trimester and there was no correlation between the change in each hormone from the second to the third trimester for individual subjects. This suggests that although the production of both hormones increases as pregnancy progresses, these changes are likely to be under the influence of independent stimuli.

The constancy of plasma progesterone after dietary sodium manipulation casts doubt on early findings¹⁴ of an acute change in production in response to altered sodium intake. This finding was almost certainly complicated by the use of urinary pregnanediol, which does not relate closely to progesterone levels. Our findings for progesterone are more in keeping with those of a placentally produced hormone whose primary function is one of maintaining a viable pregnancy and not that of acutely regulating sodium and potassium homeostasis. In addition, progesterone levels do not appear to be altered by rapid changes in mineralocorticoid production during pregnancy. It may be argued that free (active) hormone concentrations have altered, but it would be surprising if this were not reflected somewhat in absolute plasma levels, as occurred with aldosterone. The lack of change in plasma progesterone after dietary sodium loading is also in keeping with previous work that found the mineralocorticoid deoxycorticosterone, predominantly produced from maternal progesterone, to be unresponsive to sodium manipulation.¹⁵

There appears no doubt, from previous work, that progesterone is capable of increasing sodium excretion, probably by increasing proximal tubular rejection,¹⁶ and that it may therefore play a role in secondarily stimulating aldosterone production and affecting potassium balance. There is also little doubt that exogenously administered progesterone has an antimineralocorticoid action, a property borne out by more direct tissue receptor studies, but whether these effects can be translated to the physiologic circumstances of human pregnancy remains open to question. Our data do not suggest strong interdependence between progesterone and aldosterone in human pregnancy or a role for progesterone in the acute regulation of sodium and potassium excretion, but it is likely that progesterone exerts a constant long-term effect on potassium and sodium excretion proportional to its placental production.

Finally, in subjects on an unrestricted dietary regi-

men, there is a constancy of absolute urinary potassium excretion throughout pregnancy and post partum despite large changes in filtered potassium (from a mean of 810 mmol per day in the second trimester to a mean of approximately 580 mmol per day* in the nonpregnant state). The renal tubular function responsible for this adaptation remains unexplained.

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Volume reduction of the uterus during and soon after hysterectomy

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Gynecologists observe perceptible and variable reduction from uterine size in vivo to subsequent size in the pathology laboratory. To measure this decrease in uterine size, the thickness, breadth, and height of the corpus were measured four times: (1) as soon as the abdomen had been opened, (2) as soon as the uterine arteries had been ligated, (3) as soon as the uterus was removed, and (4) after 45 minutes in fixative. The cervical diameter and length were measured two times: (1) as soon as the uterus was removed and (2) after 45 minutes in fixative. Reduction in volume varies from 20% to 64% in different specimens. Reduction in volume is greatest in women who have uterine hypertrophy and hypermenorrhea. It is least in postmenopausal women who have atrophic uteri. (AM J OBSTET GYNECOL 1986;155:354-7.)

Key words: Uterine volume, reduction after hysterectomy

Frequently a uterine specimen that has just been removed from a patient seems much smaller than the original size in vivo. How much does uterine size actually diminish during and soon after hysterectomy? In which patients is this uterine shrinkage greatest or least? What are the probable causes? These questions prompted this study.

During the past century, there have been almost a score of studies concerning the sizes of extirpated uteri.¹⁻¹⁶ In 1962, Lewis et al.⁷ designated extirpated uteri that had fixed weights of ≥ 120 gm as hypertrophied and noted that these larger uteri contained measurably larger myometrial cells than did smaller uteri.

But how accurately does the freshly excised or fixed specimen reflect prior size or mass in vivo? Often the in vivo size or mass of the uterus causes the clinical symptoms that bring the patient to the gynecologist. No prior study could be found that measured uterine sizes in vivo and then compared those with measurements of the same freshly excised and fixed specimens.

Methods of study

Since a uterus cannot be weighed in vivo, we decided to take meticulous measurements, all performed by the senior author (B. R.), using an accurate calipers and a metric ruler and marking the uterus with a scalpel to preserve landmarks for comparison. All uteri removed

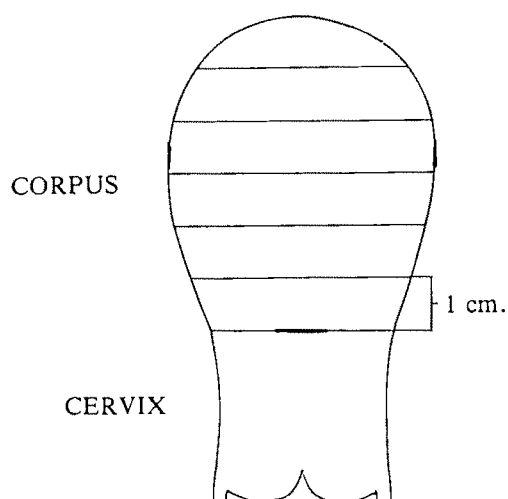


Fig. 1. The junction between the corpus and the cervix and the lateral borders of the corpus were marked with a scalpel. In each of five specimens, 1 cm sections of the corpus were measured individually.

by abdominal hysterectomy during 1984 were included, except those that were grossly distorted by large leiomyofibromas. Abnormalities that were corrected during these 37 operations, all of which included abdominal hysterectomies, are listed. In the uterus there were: eight with endometrial hyperplasia, four with endometrial polyps, three with carcinoma of the endometrium, three with adenomyosis, 15 with small myomas, 21 with hypertrophy, 19 with hypermenorrhea, 17 with secondary anemia, and six with descensus. In the vagina there were 17 with relaxation. In the cervix there were 12 with dysplasia, seven with stenosis, three with laceration-cervicitis. In the adnexa and pelvis there were seven with adhesions, 15 with endometriosis, three with

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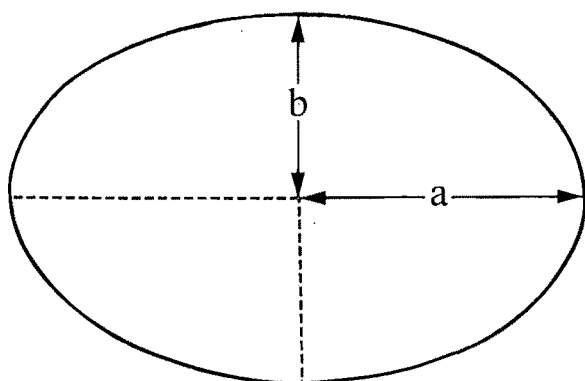


Fig. 2. A cross section of the corpus resembles an ellipse. Its area may be calculated by multiplying a times b .

dermoid cyst, three with serous cystadenoma, two with mucinous cystadenoma, five with fibroma, four with endosalpingosis, two with salpingitis, and two with hydrosalpinx.

When the abdomen was open, just before hysterectomy, a small, transverse marker incision was made anteriorly across the visible and palpable junction between the cervix and corpus of the uterus (Fig. 1). Similarly, borders were identified by observation and palpation at the widest margin of each corpus and were marked. The length, width, and thickness of the corpus of each uterus were measured four times: (1) before start of hysterectomy, (2) immediately after the major blood supply (ovarian and uterine arteries) had been cut and ligated, (3) immediately after the uterus was removed, and (4) after 45 minutes in fixative (10% formalin). The length and diameter of each cervix were measured twice: (1) immediately after the uterus was removed and (2) after 45 minutes in fixative. In several instances, the average diameter of the cervix was calculated from two or three measurements.

The volume of the essentially cylindrical cervix was calculated with the formula: Radius squared $\times$ 3.1416 $\times$ length = volume.

The volume of the essentially elliptical corpus (Fig. 2) was evaluated tentatively, with the "uncorrected" formula: $a \times b \times 3.146 \times \text{length} = \text{volume}$. Since the corpus is described as "pear-shaped" (Fig. 1), 1 cm sections from each of the first five extirpated, fresh uterine corpora were individually measured. Volumes of respective sections were calculated and were added together to determine the "corrected" volume of each corpus. In these five specimens, the average "corrected" volume was only 93% of the average "uncorrected" volume. Therefore, the volume of each subsequent corpus was calculated, with the "corrected" formula: $a \times b \times 3.1416 \times \text{length} \times 0.93 = \text{volume}$.

The four calculated volumes for each corpus and the two calculated volumes for each cervix were fed into

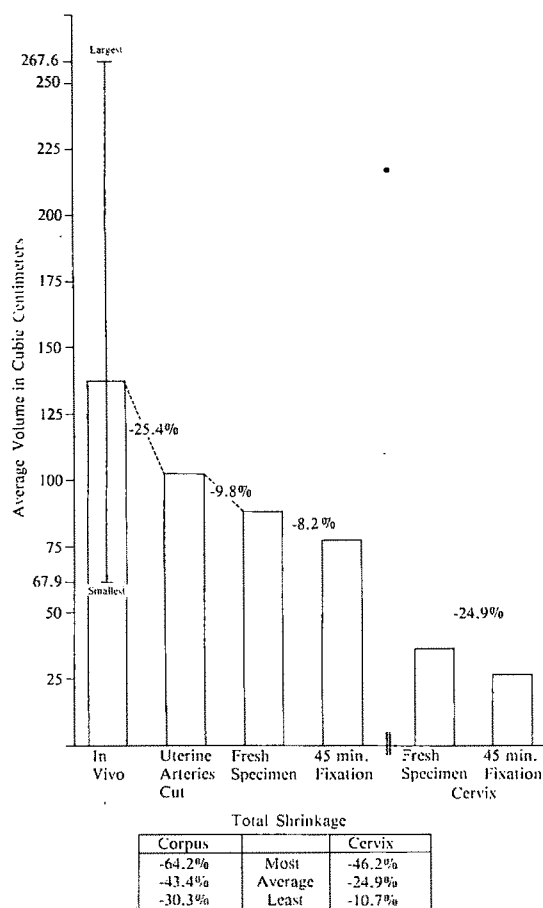


Fig. 3. Shrinkage of uterus during and after hysterectomy in 30 women of menstrual age (range 28 to 55). All reductions in volume are highly significant ($p < 0.001$).

an IBM computer. A t test for correlated samples (repeated measurements) was used to evaluate the significance of the respective average volume reductions.

To test these volume calculations by another method, the volumes of five uteri (both the fresh and fixed specimens) were evaluated by cleanly removing adnexa, by immersing corpus and cervix in water in a calibrated vessel, and by measuring the water displacement. Calculated volumes and immersion volumes were very similar, although the calculated volumes were slightly smaller (from 0.5% to 4.5% smaller) than immersion volumes.

Results

The largest total reduction in volume of the uterine corpus was 64.2%. This occurred in the hypertrophic uterus of a 51-year-old patient who had severe hypermenorrhea, causing secondary anemia; 92% of this reduction in size occurred while arteries and pedicles were being clamped, cut, and tied. This reduction probably represents loss of blood, lymph, and serum from the highly vascular uterus due to "back bleeding."

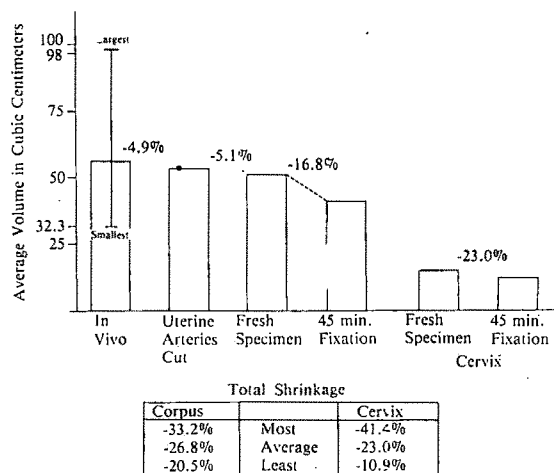


Fig. 4. Shrinkage of uterus during and after hysterectomy in seven postmenopausal women (aged 51 to 82). Probably because of the small sample, calculations did not show volume reduction between *In Vivo* and *Uterine Arteries Cut* to be significant ($p = 0.159$). Volume reduction between *Uterine Arteries Cut* and *Fresh Specimen* was significant ($p = 0.010$). Volume reduction between *Fresh Specimen* and *45 min. Fixation* was highly significant ($p < 0.001$).

Conversely, the smallest total reduction in volume of a uterine corpus was only 20.5%. This occurred in the small, atrophic uterus of a postmenopausal patient; 78.9% of this shrinkage occurred during the first 45 minutes the specimen was in fixative. It probably represents the effect of fixation on uterine connective tissues.

Cervical volumes could not be measured in vivo but could be measured (1) in the freshly extirpated specimen and (2) after 45 minutes in fixative. Fixation resulted in an average reduction in volume of 24.9% in women of menstrual age and of 23% in postmenopausal women. One cannot measure directly but can only deduce the effects of vascularity on the volumes of cervixes in vivo under variable clinical conditions.

Fig. 3 shows the averages and extremes of uterine shrinkage during and after hysterectomies that were performed in 30 women of menstrual age. An average volume reduction of 25.4% occurred while ovarian and uterine blood supplies were being severed. Additionally, an average volume reduction of 9.8% occurred while the cervical blood supplies were being severed. There was an average reduction in volume of the corpus of only 8.2% during the first 45 minutes in fixative. Each of these average reductions in volume are highly significant ($p < 0.001$).

Conversely, Fig. 4 shows averages and extremes of uterine shrinkage during and after hysterectomies performed in seven postmenopausal women. An average reduction in volume of only 4.9% occurred while ovarian and uterine vessels were being severed. Probably

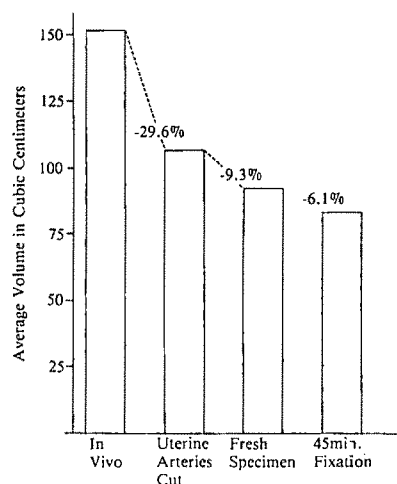


Fig. 5. Shrinkage of uterine corpus in 17 women with hypermenorrhea and secondary anemia. All reductions in volume were highly significant ($p < 0.001$).

because of the small size of the sample, calculations did not show this difference to be significant ($p = 0.159$). An average reduction in volume of only 5.1% occurred while the cervical vessels were being severed (significant, $p = 0.010$). However, an average reduction in volume of 16.8% occurred when the fresh specimens were placed in 10% formalin for 45 minutes ($p < 0.001$).

Each uterine specimen was also weighed on a metric scale (1) as soon as it was removed and (2) after 45 minutes in fixative. In addition, metric weights were recorded in the pathology laboratory after more complete fixation. Weights of the freshly excised specimens were reduced an average of 7.1% by fixation.

At each end point where comparisons could be made, measured volumes (cubic centimeters) were less than weights (grams). For instance, the average volume of all freshly excised uteri, including corpus and cervix, of 117.3 cm³ was only 86.8% of the average weight of 135.1 gm. One may calculate that 1 cm³ of freshly excised uterus and cervix weighs about 1.15 gm. Similar calculations reveal that 1 cm³ of uterus and cervix that has been fixed for 45 minutes weighs about 1.23 gm.

Since blood weighs between 1.053 and 1.055 gm/ml, a uterus that lost 100 ml of blood during hysterectomy should have lost more than 105 gm.

Using these figures and comparing them with the extremes of precise volume measurements and weights from specific patients, one may calculate that a freshly excised specimen of uterus and cervix, which now measures 100 cm³ and weighs 115 gm, may have measured in vivo as much as 244 cm³ and weighed as much as 280 gm or may have measured as little as 107 cm³ and weighed as little as 123 gm.

Further calculations disclose that a fixed specimen,

which measures 100 cm³ and weighs 123 gm, may have measured in vivo as much as 306 cm³ and weighed as much as 377 gm or may have measured as little as 154 cm³ and weighed as little as 174 gm.

Fig. 5 shows that those 17 women whose preoperative findings included uterine hypertrophy and hypermenorrhea severe enough to cause secondary anemia (ages 39 to 56 with hematocrits of 28% to 37%) had a notably greater average loss of volume of the corpus while ovarian and uterine vessels were being severed (average 29.6%, $p < 0.001$; range 45.1% to 16.3%). One may deduce (but cannot measure) a similar reduction in size of the cervix while paracervical vessels were being severed. Only five of these women had microscopic adenomyosis, and only one had a submucous fibromyoma. However, all had myometrial and cervical hypertrophy, producing an average calculated in vivo volume of uterus and cervix of 205 cm³ and an average calculated in vivo weight of 236 gm (range, from a high volume of 340 cm³ or a weight of 392 gm down to a low volume of 132 cm³ or a weight of 151 gm).

Comment

The methods of measurement and volume calculation used in this study were the only methods that could be used before and during hysterectomy, and they were used after hysterectomy for comparison. Small variations in linear measurements could produce significant modifications of volume calculations, so measurements were meticulously precise, to the millimeter. This experiment was designed to evaluate the in vivo volumes of the uterine corpus before hysterectomy and the percentages of reduction in volume of the corpus and cervix that were associated with hysterectomy and tissue fixation.

In 1975, Piironen and Kaihola,⁸ of Turku, Finland, used B-mode ultrasound to measure the sizes of uteri in 16 nonpregnant, healthy women. They found that uterine volume increased about 1.6 times between the twelfth day and the twenty-seventh day of the menstrual cycle. More detailed ultrasonic methods might give reasonably accurate in vivo uterine volumes among those patients in whom pelvic examinations are not satisfactory (for instance, obese, rigid, or uncooperative patients). However, technical errors such as overfilling the bladder could squeeze and narrow the thickness of the adjacent uterus, thus reducing measurements.

It is noteworthy that three elderly women who had Stage I carcinoma of the endometrium (ages 79, 80, and 81) had an average reduction of volume of the

corpus of 19% during hysterectomy. Conversely, the average shrinkage of the corpus during hysterectomy for all other postmenopausal patients was barely 4%. The cancerous uteri were more vascular.

All uteri shrink during and soon after hysterectomy. Large, highly vascular uteri shrink the most, and small, atrophic, hypovascular uteri shrink the least. Measurements or weights of either a fresh or a fixed specimen of the uterus bear an extremely inaccurate and highly variable relationship to the true in vivo measurements or "mass" before hysterectomy.

The probable chief causes of uterine shrinkage during hysterectomy are (1) loss of blood, lymph, and serum as respective vessels are severed and (2) contraction of hypoxic myometrial cells. After hysterectomy, fixation causes variable shrinkage of all tissues.

Criteria for uterine size should be based on the pathophysiology in vivo, not on the variably shrunken, freshly excised or fixed specimen. Methods for obtaining in vivo measurements of the uterus are improving.

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Evidence of short-loop inhibition of decidual prolactin synthesis by decidual proteins

Part I

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Induction of uterine endometrial prolactin synthesis is dependent on progesterone-induced decidualization of stromal cells. These decidual cells are not dependent on progesterone for continued prolactin synthesis. The factors modifying decidual prolactin synthesis remain largely unknown. To test the hypothesis that a decidual protein is the major modulator of new prolactin synthesis, decidua were cultured within dialysis membranes allowing the accumulation of proteins >12,000 molecular weight in a metabolically neutral environment, and the rate of new synthesis was compared with prolactin synthesis from samples cultured in 10 times the available volume for protein distribution. The rate of new prolactin synthesis at 48-hour intervals up to 144 hours was compared. Initial and postculture decidual prolactin content was obtained and was found not to vary significantly between groups ($0.05 < p < 0.10$). At 48 hours significant suppression of decidual prolactin synthesis was apparent ($p < 0.05$) within the dialysis membranes. As prolactin concentration increased during in vitro culture this suppression was enhanced ($p < 0.005$). Gel chromatography and immunoprecipitation of iodine 125-labeled prolactin added at time 0 revealed no significant degradation of the ^{125}I -labeled prolactin and maintenance of its immunoactivity even at 144 hours. This confirms that the plateauing of prolactin concentration within the dialysis membranes is due to suppression of new synthesis rather than metabolism of previously synthesized prolactin. (AM J OBSTET GYNECOL 1986;155:358-62.)

Key words: Prolactin synthesis, decidual proteins, gel chromatography, immunoprecipitation

Prolactin is synthesized and secreted by human endometrium from day 23 of the normal menstrual cycle until menses or parturition.^{1,2} This prolactin is identical to human pituitary prolactin by immunologic³ and biologic activity⁴ and various chemical criteria.⁵ The initiation of prolactin synthesis is progesterone dependent and correlates with the onset and extent of endometrial stromal decidualization.^{2,5,6} The induction of prolactin synthesis and decidualization is partially inhibited by estradiol.³ However, once decidualization and prolactin synthesis have been established, continued prolactin synthesis occurs in the absence of either hormone for extended periods of in vitro culture.⁷ The prolactin

synthesized by decidua is transported across the chorioamniotic membrane and remains biologically active throughout its existence.⁸ Factors normally associated with pituitary prolactin synthesis have not been demonstrated to influence decidual prolactin synthesis or release in vitro. While arachidonic acid and perhaps a decidual polypeptide may modulate prolactin release for short periods during in vitro culture,⁹ the importance of these factors in regulating prolactin synthesis and release during the course of 9 months of gestation is unclear. Since nearly all of the previous in vitro work on the control of decidual prolactin synthesis has occurred at tissue and media prolactin concentrations far below those occurring in vivo and/or in short-term culture, an in vitro model was devised that was capable of establishing high prolactin and decidual protein levels in media without exposing the decidua to metabolic stress. With this model the possibility of a decidual product short-loop feedback on decidual prolactin synthesis was tested.

Material and methods

Human decidual tissue was obtained from pregnancies between 37 and 40 weeks' gestation with delivery

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by cesarean section, which was not preceded by either labor or ruptured membranes. Permission to obtain these tissues was granted by the Human Investigation Committees of the University of Connecticut Medical School and Saint Francis Hospital and Medical Center. The decidua was stripped from the underlying chorion and prepared by previous published techniques.⁷ Prolactin assays were performed by a modification⁸ of the method of Sinha et al.¹⁰ with materials provided by the National Institutes of Health. Intra-assay variability was 6%. The interassay variability of 11% did not affect results since all samples from an experiment were analyzed in the same assay. Protein determinations were performed by the method of Lowry et al.¹¹ The medium was prepared as previously published⁷ and contained no hormones, additional proteins, or serum. All samples were cultured at 37° C in a metabolic shaking bath. Data were analyzed by two-directional analysis of variance to account for tissue variability and significance defined as $p < 0.05$ by the Student-Newman-Keuls test.

Experiment 1. Decidua ($N = 4$) were divided into 300 mg samples ($n = 18$) and cultured for 6 days in the following conditions. Control A (no sac—medium change) ($n = 2$) consisted of 300 mg of decidua in 30 ml of medium; the medium was sampled and changed every 48 hours. Control B (no sac—no medium change) ($n = 2$) consisted of 300 mg of decidua in 30 ml of medium; the medium was sampled every 48 hours but not changed. Experiment A (sac—medium change) ($n = 6$) consisted of 300 mg of decidua with 3 ml of medium in a dialysis membrane (VWR 25225-226) suspended in 27 ml of medium (Fig. 1). The external medium was changed every 48 hours and a pair of dialysis sacs was discontinued. The medium inside the dialysis membrane was frozen and saved for subsequent prolactin assay. Samples of medium from outside the dialysis membrane were likewise frozen and saved for subsequent prolactin assay with each change in medium. Experiment B (sac—no medium change) ($n = 6$) consisted of 300 mg of decidua with 3 ml of medium in a dialysis membrane (VWR 25225-226) suspended in 27 ml of medium. A pair of dialysis membranes was discontinued every 48 hours. The external medium was not changed but was sampled every 48 hours for subsequent prolactin assay. The dialysis membrane nominal exclusion limit is 12,000. Samples of 300 mg of tissue ($n = 2$) from each decidua at time 0 were frozen for subsequent homogenization and prolactin assay. When discontinued from culture, each sample was likewise frozen for subsequent homogenization and prolactin assay.

Experiment 2. Decidua ($N = 2$) were prepared for culture as in experiment 1, except that 1000 cpm/ml of freshly G-100 chromatographed iodine 125-labeled

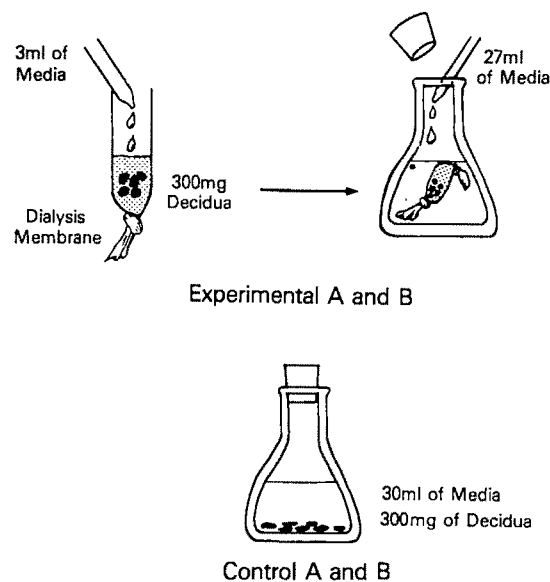


Fig. 1. In experiments A and B 300 mg of decidua is suspended in 3 ml of medium within a dialysis membrane. The membrane in turn is suspended in 27 ml of medium in a 50 ml flask. These samples along with 300 mg explants in 30 ml of medium in a 50 ml flask (controls A and B) were then cultured in a metabolic shaking bath at 37° C.

human prolactin¹² was added to the 3 ml of medium in each dialysis membrane of the experimental groups. At each 48-hour interval samples from the discontinued dialysis membranes were analyzed for the percentage of ¹²⁵I remaining in the dialysis membrane sac. Gel chromatography of 2 ml of membrane medium was performed on a Sephadex G-100 1.5 × 80 cm column as previously described.¹² Immunoprecipitation of prolactin from the column fractions was performed with prolactin antibody at a 1:400 dilution. This concentration of prolactin antibody was previously shown to precipitate 80% of prolactin at the concentration of prolactin found in the column fractions.¹² Prolactin concentrations in the media were also obtained and combined with the data in experiment 1 (bringing the number to six).

Results

At no time during any experiment was significant prolactin detected in any medium outside of the dialysis membranes. Recovery of medium from within the dialysis membranes was consistently between 2.5 and 2.8 ml. All calculations of total prolactin released into the medium were based on a potential recoverable volume of 3.0 ml, the difference being assumed to be unrecoverable because of adherence to folds in the membrane. If the decreased volume recovery was actually due to a real decrease in volume, then this assumption will have led to an overestimation of prolactin syn-

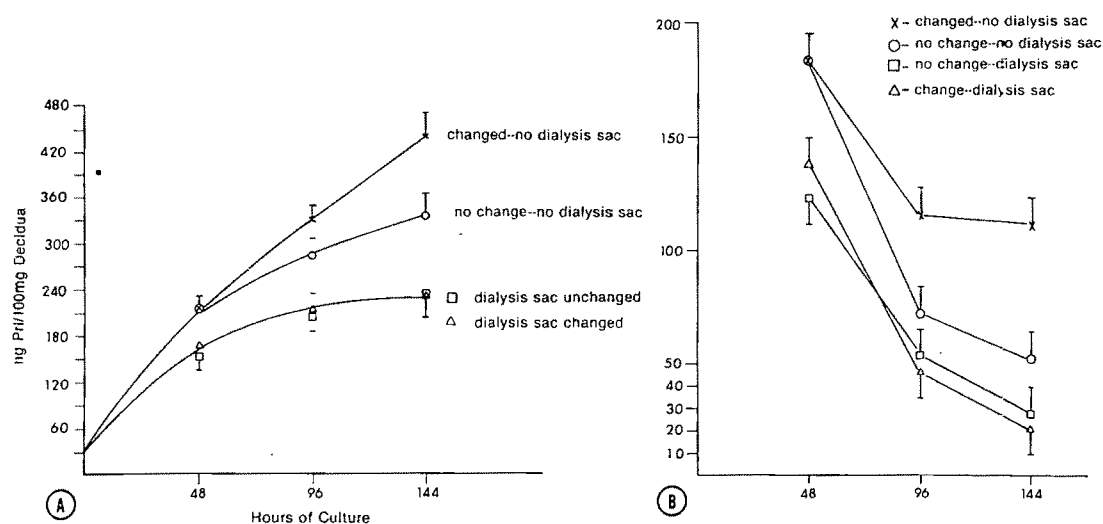


Fig. 2 A, Total prolactin synthesis in dialysis membrane samples is significantly suppressed at each time interval in comparison with control (no dialysis sac) decidua ($n = 6$). Prolactin concentration in the medium within the membranes averaged 168 ng/ml at 48 hours and 212 ng/ml at 144 hours. B, Prolactin synthesis is significantly suppressed at each 48-hour sampling interval within the dialysis sacs. Suppression in the unchanged-nondialysis sac samples occurred at 96-hour ($p < 0.10$) and 144-hour ($p < 0.05$) intervals.

thesis and release into the medium within the membranes. Since the experiments revealed a significant degree of suppression of prolactin synthesis and release within the dialysis membranes, the degree of suppression will have been underestimated.

Experiment 1. The initial prolactin content of these decidua ($n = 6$) varied from 17 to 39 ng/100 mg of tissue. The content of prolactin in samples discontinued from the control culture environments averaged 50% of the initial decidual prolactin content but varied markedly. Likewise there was marked variation in the decidual prolactin content of the samples discontinued from within the dialysis membranes, although these were invariably between 60% and 100% of the initial prolactin content. The difference in decidual prolactin content between dialysis membrane samples and control samples was not significant, $0.05 < p < 0.10$, because of the marked variation. Since the prolactin in the tissue represented $< 5\%$ of the total prolactin synthesized in any 48-hour time period and was not statistically different between sample groups, it was not included in subsequent data analysis of prolactin synthesis.

At 48 hours significant suppression of prolactin synthesis and release from decidua contained within the dialysis membranes was observed ($p < 0.05$). At 96 and 144 hours this suppression persisted in both the changed and nonchanged dialysis membrane groups ($p < 0.01$). A trend toward suppression of prolactin synthesis in control B at 96 hours ($p < 0.10$) was observed and the difference became significant at 144 hours ($p < 0.05$) (Fig. 2, A and B). No difference in suppression was noted between experiment B and

experiment A. The total prolactin synthesized by 144 hours in control B was consistently less than in control A ($p < 0.05$) and was markedly suppressed in the decidua cultured within the dialysis membranes ($p < 0.001$). The pH in control B was between 7.2 and 7.3 in most samples at 144 hours, indicating only mild metabolic stress.

Analysis of selected samples from several experiments indicated that total protein concentration within the dialysis membranes was higher than the protein concentration within the media of control samples, although the data were not extensive enough to establish a comparison between general protein synthesis and prolactin synthesis.

Experiment 2. The recovery of ^{125}I -labeled prolactin from within the dialysis membrane was consistently 80% to 90%, indicating that the ^{125}I -labeled prolactin was not being degraded into fragments that could traverse the membrane. Recovery of ^{125}I activity in medium surrounding the membrane sacks was negligible. Chromatography revealed that the ^{125}I migrated in the same location as decidual prolactin (Fig. 3). Immunoprecipitation with antiprolactin antibody resulted in an 80% recovery of the ^{125}I activity from the region of the prolactin peak. These data indicate that prolactin was not being degraded into smaller peptides and that the prolactin maintained its antigenic activity during prolonged in vitro culture.

Comment

These experiments demonstrate that prolactin synthesis is markedly inhibited during extended periods by specific products of decidual secretory activity.

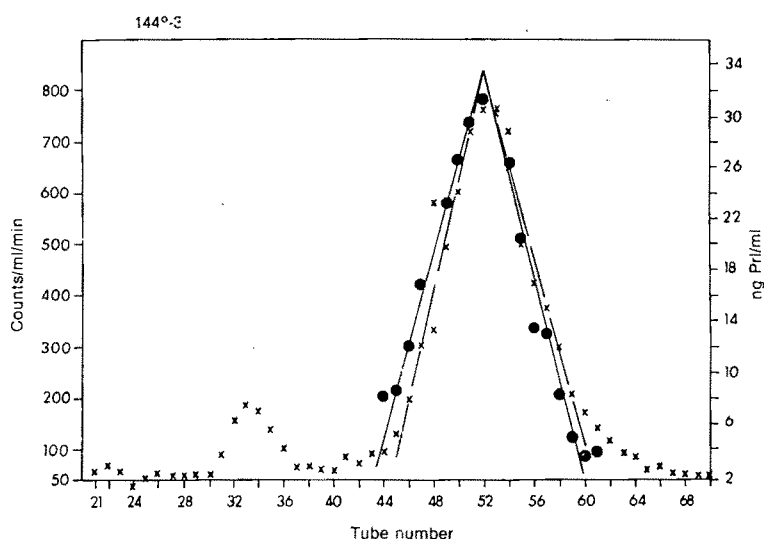


Fig. 3 ^{125}I recovery after 144 hours of culture is conserved in the prolactin peak by Sephadex G-100 chromatography. Recovery of 80% to 90% was uniformly achieved. This ^{125}I -labeled prolactin remained immunoprecipitable (x—x, nanograms of prolactin per milliliter; ●—●, immunoprecipitable ^{125}I , counts per milliliter per minute, prolactin antibody at 1:400 dilution).

This suppression is not due to low molecular weight metabolic factors. Since suppression of prolactin synthesis was significantly greater within the dialysis membranes when compared with either the changed control A or more important the unchanged control B it must be concluded that specific high molecular weight (>12,000) protein(s) or protein-bound substances are responsible for the suppression. Further the relatively mild metabolic changes occurring in control B and the lack of additional suppression in experiment B compared with experiment A suggest that the relative prolactin suppression of control B as compared with control A is due to the same specific factor(s) as in the dialysis membrane samples rather than being due to metabolic changes. However, the prolactin synthesis suppression would be less marked since the suppressive factor(s) would be at a lower concentration when diluted in 30 ml of volume instead of the 3 ml in the dialysis membranes.

The relative, although nonsignificant, increase of prolactin content in the decidua within the dialysis membranes at the conclusion of culture is also suggestive of an increase in prolactin storage within the decidua when media concentrations of prolactin are high. Further, the low level at which new prolactin synthesis occurred between 96 and 144 hours is consistent with the low level of prolactin turnover normally found in amniotic fluid.¹³ Since the concentration of prolactin in the media at this time approached the initial tissue content of these decidua, it is suggestive that the normal physiologic steady state of decidua in vivo is a high tissue prolactin content in equilibrium with a high amniotic fluid prolactin concentration but with the new prolactin synthesis rates being low.

The maintenance of ^{125}I -labeled prolactin antigenicity and molecular weight is confirmation that it is the process of new prolactin synthesis and release that is slowed and that the decrease in prolactin accumulation is not due to increased degradation. While it is possible that the bioactivity of the prolactin might be lost with time this does not seem to be the case in other in vitro models of decidual synthesis.⁸ Unanswered by this study is whether the decidual samples cultured in the dialysis membranes are capable of the same uninhibited prolactin synthesis and release when removed from the membrane as samples never confined to the dialysis membranes and what product(s) of decidual synthesis actually account for prolactin suppression. Some of this work has been completed and is reported in the accompanying article.

This experimental model suggests that products of decidual synthesis are the primary modulator(s) of decidual cell prolactin synthesis and that the baseline of prolactin-decidual cell interaction is high prolactin content—low prolactin synthesis. Since nearly all previous work on the control of prolactin synthesis has been performed in low prolactin content—high prolactin synthesis environments the results of those experiments may be misleading and have no relevance to normal decidual prolactin physiologic features.

I am deeply indebted to the obstetric delivery nurses of St. Francis Hospital and the University of Connecticut Medical School for helping to collect specimens, to Nilka Soto-Albors and Jill Bates without whose help in the laboratory the experiments could not have been performed, and to Myrtle Bacon in the preparation of this manuscript. I would also like to express my appreciation to my colleagues, Clifford Walters and Carlos

Soto-Albors, whose critical appraisals helped sharpen the focus of these experiments.

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Evidence of short-loop inhibition of decidual prolactin synthesis by decidual proteins

Part II

Douglas C. Daly, M.D., Sally Kuslis, M.D., and Daniel Riddick, M.D.

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We have recently demonstrated the suppression of decidual prolactin synthesis by decidual proteins accumulating in a dialysis membrane. In this report the reversibility of this suppression and the molecular weight range of the suppressor(s) are examined. Samples of 300 mg of decidua ($n = 10$) were cultured in three environments: (1) in 30 ml of medium (control), (2) in 3 ml of medium in a dialysis membrane suspended in 27 ml of medium, and (3) in 2.7 ml of medium with 0.3 ml of concentrated decidual proteins of 10,000 to 35,000 molecular weight (giving a time 0 prolactin concentration of 220 ng/ml) in a dialysis membrane suspended in 27 ml of medium ($n = 5$) or in 2.7 ml of medium with 0.3 ml of concentrated decidual proteins $>35,000$ molecular weight (giving a time 0 prolactin concentration of 5 ng/ml) in a dialysis membrane suspended in 27 ml of medium ($n = 5$). Dialysis membranes were discontinued at 48, 96, and 144 hours and the decidua within was subsequently cultured in 30 ml of medium until 240 hours. The suppression of prolactin synthesis identified previously was confirmed in this study. Further it was found that the suppression of decidual prolactin synthesis was reversed by the removal of decidua from the high decidual protein-prolactin environments of the dialysis membranes. Third, only the addition of decidual proteins between 10,000 and 35,000 molecular weight with a high prolactin concentration enhanced the suppression of new prolactin synthesis. We conclude that a decidual protein(s) between 10,000 and 35,000 molecular weight, possibly prolactin itself, is the primary control factor(s) in the modulation of prolactin synthesis in term decidua. Further the normal physiologic state of decidua is a high prolactin concentration in equilibrium with low prolactin synthesis. Therefore studies intended to assess the effects of modulators of prolactin synthesis in decidua should not be performed in low prolactin concentration-high prolactin synthesis conditions during short time periods (as has been the case in a majority of studies to date) since such conditions do not reflect normal physiologic conditions and may result in invalid conclusions (AM J OBSTET GYNECOL 1986;155:363-8.)

Key words: Decidual prolactin synthesis, decidual proteins, dialysis membrane

The factors that modulate prolactin synthesis in decidualized endometrium have remained elusive to our understanding. Prolactin synthesis is initiated by progesterone-induced decidualization of proliferative endometrium¹ and correlates with the extent of the decidualization in both normal and pathologic condi-

tions.^{2,3} However, once transformed to decidua, progesterone is not necessary for prolactin synthesis in extended in vitro culture.⁴ Attempts to modulate prolactin synthesis from mature decidua have shown no response to factors known to modulate pituitary prolactin synthesis or release.⁵ Some studies have suggested that arachidonic acid and/or its prostaglandin derivatives⁶ and a low molecular weight decidual peptide⁷ may have transient suppressive effects on prolactin synthesis during in vitro culture when prolactin in the medium is at a low concentration. More recently we have demonstrated that decidual proteins with a molecular weight greater than 12,000 have a marked and sustained inhibitory effect on decidual prolactin synthesis and release.⁸ In this report the reversibility of this suppression of decidual prolactin synthesis is assessed and initial experiments to define the factor(s) that result in this suppression are examined.

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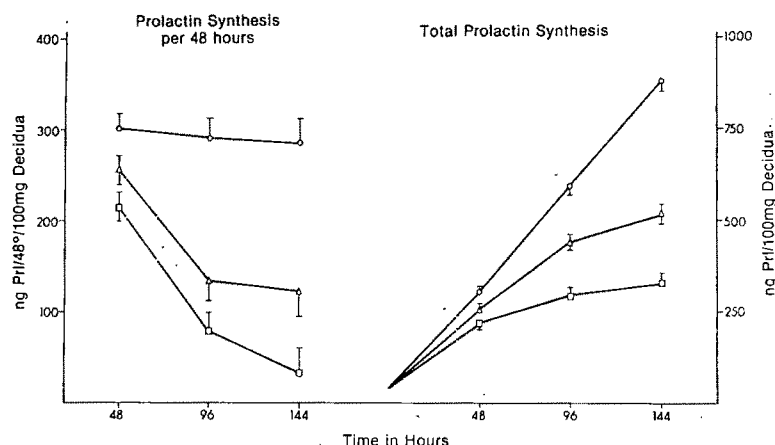


Fig. 1. Prolactin synthesis during 144 hours was markedly suppressed when decidua ($n = 5$) was cultured within dialysis membranes (Δ — Δ) when compared with control values ($\circ$ — $\circ$). Further suppression occurred with the addition of prolactin (220 ng/ml) containing decidual proteins with a molecular weight between 10,000 and 35,000 ($\square$ — $\square$; ($p < 0.05$ at 48 hours, $p < 0.01$ at 96 and 144 hours). The net decrease in total prolactin synthesis between decidua only (Δ — Δ) and decidua plus decidual proteins ($\square$ — $\square$) approximated the prolactin added at the start of the experiment ($520 - 310 = 210$ ng/ml of prolactin versus 220 ng/ml of prolactin added).

Material and methods

Human decidual tissue was obtained from pregnancies between 37 and 40 weeks' gestation and delivered by cesarean section, which was not preceded by either labor or ruptured membranes. Permission to obtain these tissues was granted by the Human Investigation Committees of the University of Connecticut Medical School and Saint Francis Hospital and Medical Center. The decidua was stripped from the underlying chorion and prepared by previously published techniques.¹ Prolactin assays were performed by a modification¹ of the method of Sinha et al.⁹ with materials provided by the National Institutes of Health. Intra-assay variability was 6%. Inter-assay variability of 11% does not affect results since all samples from an experiment were analyzed in the same assay. Protein determinations were performed by the method of Lowry et al.¹⁰ The medium was prepared as previously published¹ and contained no hormones, additional proteins (except as described below), or serum. All samples were cultured at 37° C in a metabolic shaking bath in 50 ml flasks. Data were analyzed by two-way analysis of variance and significance was defined as $p < 0.05$ by the Student-Newman-Keuls test.

Preparation for experiments 1 and 2. Minced decidua, 1 to 2 gm, from cesarean section deliveries was cultured in 250 to 500 ml of medium for 72 to 86 hours and pooled. The pooled material was concentrated 1:20 over a PM-10 amicon membrane (nominal exclusion limit 10,000). Samples of 50 ml of this concentrate underwent chromatography on a 2.5×100 cm Sephadex G-100 column, and fractions equivalent to 10,000 to 35,000 and $>35,000$ molecular weight were pooled and

concentrated. The concentration of prolactin in the 10,000 to 35,000 molecular weight fraction was 2200 ng/ml. The prolactin concentration in the $>35,000$ fraction was 50 ng/ml. The protein concentrate of the 10,000 to 35,000 fraction was one fifth that of the $>35,000$ fraction and averaged 200 μ g/ml; the protein concentrate of the $>35,000$ fraction averaged 1.0 mg/ml before dilution in experiments 1 and 2.

Experiment 1. The decidua ($N = 5$) was prepared as described previously.¹ The control ($n = 3$) was 300 mg of decidua in 30 ml of medium with samples obtained and medium changed every 48 hours for 10 days. The experimental group A ($n = 6$) was 300 mg of decidua in 3 ml of medium within the dialysis membrane suspended in 27 ml of medium. Two membranes were discontinued every 48 hours. Samples of medium were obtained from each membrane when discontinued and frozen for future assay. All external media were sampled and frozen for subsequent assay every 48 hours. The decidua was extracted from each discontinued membrane and cultured until day 10 in 30 ml of medium, the medium being sampled and changed every 48 hours. The experimental group B ($n = 6$) was treated identically to experimental group A except that the 10,000 to 35,000 concentrate of decidual proteins including prolactin was added to the medium placed in the dialysis membrane at a 1:10 dilution with the total volume being 3 ml. The prolactin concentration in the dialysis membrane was 220 ng/ml at the start of the incubation. Initial decidual prolactin concentrations were determined on homogenized decidua from time 0.¹ The concentration of prolactin in decidua after culture was not determined since it was

previously shown that it represents <5% of new prolactin synthesis in this experimental model.⁸

Experiment 2. Decidua (N = 5) was prepared and cultured in an identical manner to experiment I except for the experimental group B. The medium in the dialysis membranes of these samples contained a 1:10 dilution of the protein fraction >35,000 with a final prolactin concentration of 5 ng/ml. The decidua was recovered from each dialysis membrane when discontinued and cultured until day 10 when the experiment was terminated. The medium was sampled and changed every 48 hours. Initial decidual prolactin concentrations were determined on homogenized decidua from time 0.

Results

At no time during any experiment was prolactin detected in any medium outside of the dialysis membranes. Recovery of medium from within the dialysis membranes was consistently between 2.4 and 2.8 ml. All calculations of total prolactin released into the medium were based on a potential recoverable volume of 3.0 ml, the difference being assumed to be unrecoverable because of adherence to folds in the membrane. If the decreased recovery was actually due to a real decrease in volume then this assumption will have led to an overestimation of prolactin synthesis and release within the membrane sacs and therefore an underestimation of the degree of suppression occurring.

Experiment 1. Suppression of prolactin was noted as in the previous study⁸ at 48 hours ($p < 0.05$) and continued to an even greater extent at 96 and 144 hours ($p < 0.005$) (Fig. 1) in both experimental groups A and B. Once removed from the dialysis membranes the majority of the decidua samples demonstrated a recovery of decidual prolactin synthesis in the subsequent time periods, although the prolactin production did tend to lag behind the prolactin production from control decidua of the same duration of culture (Fig. 2). This difference was not significant after samples had been removed from the dialysis sacs for more than 48 hours. In the time periods 144 to 192 hours and 192 to 240 hours prolactin synthesis was accelerated in all samples when compared with that of previous time intervals ($p < 0.05$), suggesting an increasing capacity of the decidua to synthesize prolactin when chronically exposed to a low prolactin-low protein medium. The failure of the 48 hour samples of experimental group B to rebound is probably accounted for by two samples from separate decidua that ceased making significant prolactin. Unfortunately the decidua had not been saved when discontinued from culture so that histologic assessment was not possible. These were the only two samples demonstrating this pattern. When these two samples are eliminated from the data the pattern of

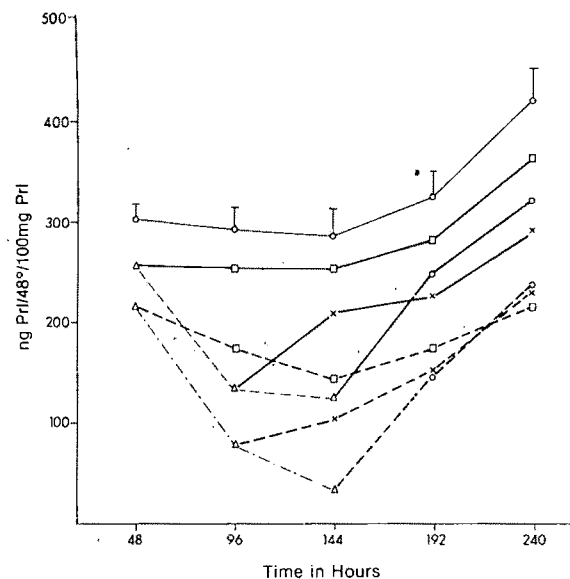


Fig. 2. Prolactin synthesis was again suppressed when decidua was cultured within dialysis membrane sacs (Δ — Δ) when compared with control decidua ($\circ$ — $\circ$) ($p < 0.01$ at 96 and 144 hours) ($n = 6$). The suppression of prolactin synthesis was augmented by addition of decidual protein concentrate, in a molecular weight range of 10,000 to 35,000, with a final prolactin concentration of 220 ng/ml, to the dialysis membrane sacs (Δ — $\square$) ($p < 0.05$ at 48 hours, $p < 0.01$ at 96 and 144 hours). This suppression was largely reversible as demonstrated by the increase in prolactin synthesis after decidua was removed from the dialysis sac at each time period (Δ — $\square$, Δ — $\times$, Δ — $\circ$, decidua — membrane at 48, 96, and 144 hours, respectively) (Δ — $\square$, Δ — $\times$, Δ — $\circ$, decidua plus protein — membrane at 48, 96, and 144 hours, respectively).

rebound of prolactin synthesis is seen at this time period as with the other five groups.

Samples in which proteins of 10,000 to 35,000 molecular weight including 220 ng/ml of prolactin were added to the dialysis membrane demonstrated a trend to further prolactin suppression at 48 hours ($p < 0.10$) when compared with membrane decidua without additional proteins and prolactin. This suppression became significant at 96 hours and persisted through 144 hours ($p < 0.01$) (Fig. 1). At 144 hours of culture the total prolactin concentration of the dialysis membrane medium of experiment A and experiment B were virtually identical: 520 ng/ml in the dialysis membranes with no additional protein and 530 ng/ml in the dialysis membranes in which 220 ng/ml of prolactin with protein (10,000 to 35,000 molecular weight) had been added. Therefore, the additional prolactin synthesis in the dialysis membranes of experiment A in which no additional protein-prolactin had been added was 210 ng/ml, nearly identical to the 220 ng/ml of prolactin added to the dialysis membranes of experiment B. The concentration of prolactin in the medium within the

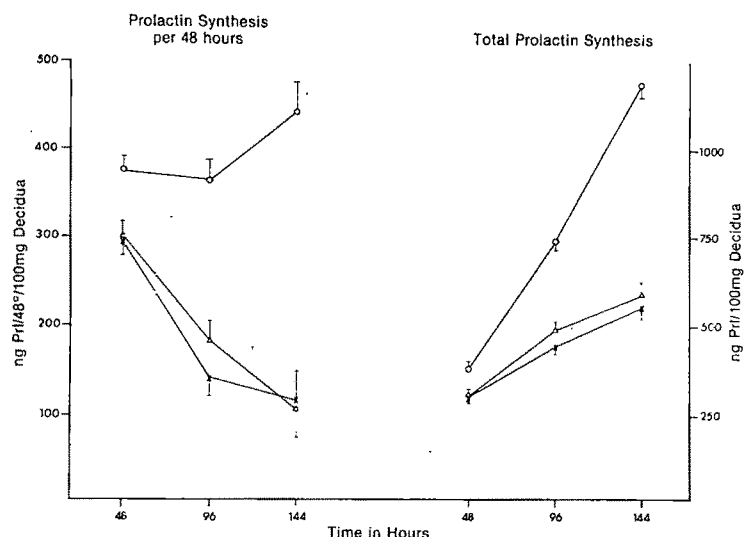


Fig. 3. In this set of experiments ($n = 5$) prolactin synthesis was again suppressed by culture within the dialysis membrane sac (Δ — Δ) when compared with the control value ($\circ$ — $\circ$). However, the addition of high molecular weight decidual proteins $>35,000$ (prolactin concentration 5 ng/ml), even with five times the total protein concentration, to the dialysis sac ($\times$ — $\times$) did not further suppress decidual prolactin synthesis.

dialysis membranes approximated the mean prolactin content of the individual decidua before culture (350 to 810 ng/gm of tissue, with a mean of 640 ng/gm).

Experiment 2. The pattern of prolactin suppression within the dialysis membranes was the same as that observed in the previous experiment. In addition, rebound of prolactin synthesis was again noted when the decidua were removed from the dialysis membranes and cultured in 30 ml of medium, thus maintaining lower prolactin concentration in the medium (data not shown). The high molecular weight-low prolactin-decidua protein mix ($>35,000$) added to decidua within the dialysis membranes of experimental group B in this experiment had no additional impact on decidual prolactin synthesis. At no time interval was any additional suppression of prolactin synthesis noted (Fig. 3).

Comment

These experiments indicate that a factor or group of factors in the same molecular weight range as prolactin has a suppressive effect on further prolactin synthesis and release during extended periods of time. This suppressive effect on prolactin synthesis is specific to the molecular weight range of 10,000 to 35,000 since suppression does not occur with decidual proteins of greater molecular weight. This suppression is reversible with a rapid increase in prolactin synthesis occurring when the suppressive factor(s) have been removed or diluted.

Three possible categories of substances could be involved in this suppression: (1) a low molecular weight

substance (such as a prostaglandin or peptide) specifically bound to a transport protein with a molecular weight of 10,000 to 35,000, (2) a nonprolactin protein with a molecular weight of 10,000 to 35,000, or (3) prolactin itself. No further identification is possible from these data but the close agreement between the additional prolactin synthesized in the experiment A samples of experiment 1 with the amount of prolactin added to experiment B samples in that experiment suggest that it may be prolactin itself that is exerting this short-loop negative feedback.

Interestingly data supporting a potential short-loop feedback effect on prolactin synthesis in the pregnant rhesus monkey were published in the mid 1970s before the demonstration of decidua as the source of amniotic fluid prolactin. Josimovich et al.¹¹ had demonstrated that the turnover rate of amniotic fluid prolactin was 1% to 2% per hour in the steady state. However, as shown in Fig. 4,¹² when 70% to 80% of the amniotic fluid volume and therefore 70% to 80% of amniotic fluid prolactin was replaced there was a rapid reaccumulation of prolactin to within 20% of the initial concentrations within hours. This reaccumulation could not be accounted for by maternal or fetal serum prolactin transfer. Josimovich et al. felt that the reaccumulation of prolactin was probably due to prolactin stored in the membranes-decidua. However the amount of prolactin stored could not by itself account for the reaccumulation. In light of the data presented here this reaccumulation most likely occurs as a result of the release of decidual prolactin synthesis from

short-loop negative feedback as prolactin or other proteins were transported across the amnion-chorion^{13, 14} to the amniotic fluid in response to the loss of prolactin and/or other proteins from the amniotic fluid. Therefore the reaccumulation is due to release of stored prolactin and rapid synthesis and transport of new prolactin.

The suppression of prolactin by short-loop feedback of a 10,000 to 35,000 molecular weight protein (or a 10,000 to 35,000 molecular weight protein-bound substance) also would explain the close correlation noted between initial tissue concentration and prolactin synthesis noted by Rosenberg et al.¹⁵ Since their experiments were performed at low prolactin concentrations in the medium, these decidua would have been synthesizing prolactin rapidly in an attempt to reestablish normal prolactin concentration in the surrounding media. The higher prolactin concentrations in some decidua would indicate a higher prolactin short-loop feedback set point. These decidua would more likely be capable of synthesizing prolactin at higher rates in an attempt to reestablish tissue-medium equilibrium. This close correlation in initial tissue prolactin concentration and prolactin synthesis seen in the experiments reported by Rosenberg et al. would also suggest that prolactin itself is the short-loop feedback inhibitor. The data of Luciano et al.¹⁶ showing that prolactin synthesis was increased by the addition of serum components could represent the partial physiologic removal of the suppressive factor by binding to serum proteins, thus lowering the functional concentration of the feedback inhibitor. This would partially release decidua from the short-loop inhibition and lead to enhanced prolactin synthesis by the decidua. Unfortunately the high cost and lack of availability of purified prolactin have kept us from directly testing the hypothesis that prolactin itself is the short-loop feedback modulator in this model.

Finally, these data will require a reassessment of the existing data on the control of decidual prolactin synthesis. It is probable that the short-loop feedback suppression demonstrated in these experiments is the basic control mechanism on which other factors affecting prolactin synthesis would interact. Therefore experiments performed on media with prolactin levels significantly below normal tissue levels may not be reflective of normal decidual physiologic features but rather may represent the response of a stressed tissue to a nonphysiologic environment. This would be particularly true of experiments testing short-term prolactin synthesis control at negligible prolactin-decidual protein concentrations in media.³⁻⁷ It is probable that a variety of substances such as steroid hormones, prostaglandins, and peptides may affect decidual prolactin synthesis by affecting the nature or sensitivity of the

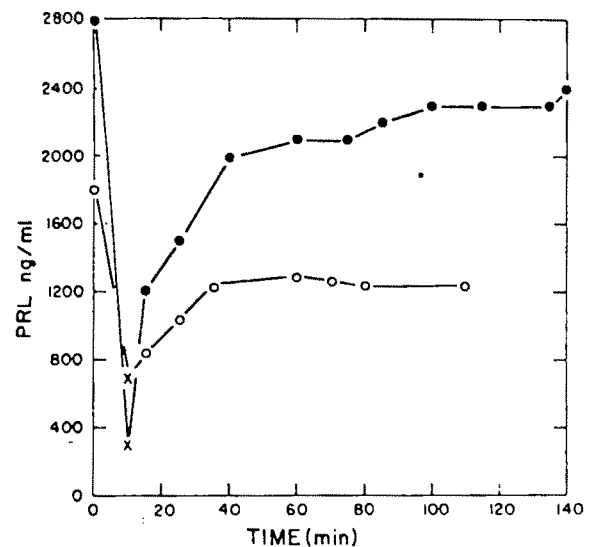


Fig. 4. Amniotic fluid prolactin in the 2 hours after 60% to 78% replacement of the fluid with prolactin-free fluid. Points designated by crosses, at 10 minutes, signify calculated nadir after amniotic fluid replacement. Symbols represent measured values of two animals. These results indicate rapid prolactin reaccumulation that can only be accounted for by an increase in new prolactin synthesis. (From Josimovich JB, Merisko K, Boccella L. Amniotic prolactin control over amniotic and fetal extracellular fluid water and electrolytes in the rhesus monkey. *Endocrinology* 1977;100:564. Copyright by The Endocrine Society.)

short-loop feedback mechanism demonstrated in these experiments. Such modifying effects could account for the fall in amniotic fluid and decidual prolactin concentrations in the third trimester. Further study is clearly needed on the control of decidual protein synthesis and particularly prolactin synthesis. The demonstration in these data of a short-loop feedback suppression of prolactin synthesis in the decidua will allow for the design of experiments to elucidate what other factors modulate decidual function.

I am deeply indebted to the obstetric nurses of St. Francis Hospital and the University of Connecticut Medical School for helping to collect specimens, to Nilka Soto-Albors and Jill Bates without whose help in the laboratory the experiments could not have been performed, and to Myrtle Bacon in the preparation of this manuscript. I would also like to express my appreciation to my colleagues, Clifford Walters and Carlos Soto-Albors, whose critical appraisals helped sharpen the focus of these experiments.

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Infected müllerian anomaly: An unusual cause of pelvic abscess during pregnancy

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The history of a pregnant woman with a pelvic abscess related to an infected müllerian anomaly is detailed. The differential diagnosis as well as management is outlined. (*AM J OBSTET GYNECOL* 1986;155:368-70.)

Key words: Pelvic abscess, pregnancy, müllerian duct

The relatively frequent occurrence of amnionitis and pyelonephritis makes it imperative that the obstetrician diagnose and manage these infectious complications during pregnancy. Fortunately, the necessity to treat a serious pelvic abscess during pregnancy is rare. However, in the latter situation it is important that the clinician establish a diagnosis and institute appropriate therapy so that maternal (sepsis) and fetal (abortion) risks are decreased. The clinical signs associated with an intra-abdominal or pelvic abscess may be atypical during pregnancy as uterine enlargement alters the anatomic location of certain organs. Additionally, the

character of associated symptoms may be masked by the normal symptoms of pregnancy. This clinical situation is further complicated by the fact that the specific diagnostic methods may carry significant risks to the fetus.

The following case report details the management of a pelvic abscess secondary to an infected müllerian anomaly.

Case report

A 19-year-old, white, nulliparous woman was referred to the University of Alabama at Birmingham with an intrauterine pregnancy of 26 weeks' gestational age. Her pregnancy had been uncomplicated until 1 week before admission when she had noted an increased amount of vaginal discharge associated with anorexia, nausea, and vomiting. She claimed the recent onset of nightly temperature elevation to 103° F.

On admission, the blood pressure was 105/70 mm Hg, and the pulse was 120 bpm. Temperature was 99.4°

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Fig. 1. A, An anteroposterior view of a hysterosalpingogram demonstrating the cephalad filling (arrows) of a müllerian remnant. B, A lateral view demonstrates the cephalad course (arrow) of the remnant.

F. A general physical examination was normal with the exception of some left lower-quadrant tenderness. There was no evidence of peritoneal irritation. The fundal height was 25 cm and the fetal heart rate was 150 bpm. During a speculum examination, a protruding left lateral vaginal fornix was noted and purulent drainage from a small pinpoint area just lateral to the cervix was apparent. There was no evidence of ruptured membranes. On bimanual examination, a 10 by 12 cm mass extending from the left lateral vaginal fornix was palpable.

At the time of admission, the white blood cell count was $7400/\text{mm}^3$. The remainder of the laboratory evaluation including serum electrolytes, coagulation studies, liver function, renal function and urinalysis was normal. Ultrasonic examination confirmed the presence of a fetus of 26 weeks' gestational age and a left pelvic mass thought to be an abscess. She was placed on a regimen of intravenous clindamycin and an aminoglycoside. The pinpoint area of spontaneous drainage in the left vaginal fornix was carefully probed with a yield of approximately 200 ml of purulent material. After 3 days, the symptoms resolved. A single-view intravenous urogram failed to delineate the left kidney or collecting system. The right kidney had mild hydronephrosis compatible with pregnancy changes. Ultrasonic examination confirmed the absence of a left kidney. After 1 week of parenteral antibiotics, the abscess had completely resolved and a repeat bimanual examination indicated only mild residual thickening on the left side of the pelvis. The patient was discharged on a regimen of oral antibiotics with an evaluation of the urinary tract scheduled after delivery.

Four weeks later, she was readmitted with fever,

chills, and premature contractions. There was no evidence of ruptured membranes. Pertinent physical findings included a normal blood pressure and pulse. The temperature was 99.8°F . There was mild left lower-quadrant tenderness, but no peritoneal irritation. Bimanual palpation indicated that the left pelvic mass had recurred. At the time of rehospitalization, she was experiencing contractions at 5-minute intervals. The fetal heart rate was judged to be reactive and there were no decelerations. The white blood cell count was $14,800/\text{mm}^3$.

The remainder of the laboratory studies was normal with the exception of a serum bicarbonate of 18. An amniocentesis was performed to eliminate the possibility of amnionitis, and 2 ml of purulent amniotic fluid was obtained. After intravenous administration of gentamicin, penicillin, and clindamycin, labor was augmented. Cervical dilatation arrested at 5 cm, and at this time, a decreased fetal heart rate baseline variability was present.

A low transverse cesarean section was performed and a 1200 gm male infant with Apgar scores of 6 at 1 minute and 8 at 5 minutes was delivered. The cord pH was 7.23. A 30% abruptio placentae was noted. During operation, a bicornuate uterus was noted. The left pelvis was markedly indurated, obscuring anatomic landmarks; however, no fluctuance or abscess cavities were palpable or visible. After operation, blood cultures grew *Escherichia coli* and *Klebsiella*, and she completed treatment with appropriate parenteral antibiotics. Cultures were obtained from the infant, and he was begun on a regimen of antibiotics but suffered apparent septic complications and died at 48 hours.

Following complete resolution of the symptomatol-

ogy, a renal scan was performed. It failed to reveal functional ectopic renal tissue. Cystoscopy indicated no left ureteral opening. A fistulogram demonstrated a fistula from the vaginal fornix that communicated with the uterus and an apparent müllerian remnant.

After delivery, the patient did well and 6 months later a hysterosalpingogram (Fig. 1) demonstrated a bicornuate uterus. A communication at the left cervical-uterine junction was apparent with cephalad filling of a müllerian remnant. Because the patient desired future fertility, an exploratory laparotomy was performed with resection of the paramesonephric tissue and a Tomkins metroplasty. The vaginal opening of this fistula appeared clinically like a cervix. Pathologic evaluation indicated the tissue to be compatible with a müllerian remnant. The postoperative course was uncomplicated and 6 months later she returned with an early intrauterine pregnancy. The clinical course during this pregnancy was uncomplicated, and she was delivered of a viable infant by cesarean section. She continues to do well although she has not obtained a subsequent pregnancy.

Comment

The rarity of serious coexisting pelvic abscess in pregnancy does not allow any one physician or institution to develop a great experience in the diagnosis and management. However, if an abscess is suspected the differential diagnosis must include any acute abdominal disease as well as complications specific to pregnancy. Although pelvic abscess remains a significant health problem in nonpregnant women, to date, there are less than 20 reported cases of a tuboovarian abscess during pregnancy. Gastrointestinal origins that must be considered as a cause of a pelvic or intra-abdominal abscess include acute appendicitis (which occurs in approximately one in 1500 pregnancies), acute cholecystitis (which occurs in one in 3500 pregnancies), inflammatory bowel disease, and ulcer. Although these occurrences are unusual, the alert clinician should attempt to establish the likelihood of a gastrointestinal source as optimal therapies may differ significantly. Potential serious urinary infections including perinephric abscess and infection proximal to an obstructing urinary calculus may mimic pelvic abscess.

Regardless of the source, the suspicion of a pelvic

abscess may necessitate the use of diagnostic radiologic procedures. Ultrasonography may demonstrate an echo-free mass with a clearly defined posterior border, which is characteristic of a pelvic abscess. Other radiologic studies may be necessary to evaluate the urinary or gastrointestinal tract. Most of these diagnostic radiologic methods deliver doses far less than the 5 to 15 rads that is considered safe to the fetus,¹ and if indicated they can be performed. Importantly, the physician must understand the potential alterations in radiologic studies (that is, physiologic hydronephrosis) related only to pregnancy and justify their use in each individual instance.

In this particular situation, pelvic infection and abscess secondary to infection of a müllerian anomaly, which probably represents improper vertical fusion of the müllerian system,² occurred with a normal white blood cell count and minimal temperature elevation. This type of infection is rare during pregnancy and in the nonpregnant woman; however, contamination of this tract occurred and recurred during this pregnancy. Vaginal epithelial changes may have mechanically "plugged" this anomaly that communicated with the vagina and allowed bacterial proliferation; however, this particular infection may have resulted secondary to an alteration in the immunologic state that occurs during pregnancy. Some combination of these possibilities are likely as the patient related no previous problems with infection of this anomaly. Importantly, a suitable site for vaginal drainage obviated the need for exploration or percutaneous drainage. An initial vaginal incision into the obstructed müllerian remnant or hemivagina was a consideration; however, the rapid clinical response to local drainage and broad spectrum antibiotics allowed the continuation of pregnancy. Unfortunately, the infection recurred and the recognition of a communication with the intrauterine contents mandated delivery.

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Acute myocardial infarction in the puerperium in patients receiving bromocriptine

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Although described in the literature as a powerful vasodilator, bromocriptine has been noted to cause vasospasm and hypertension. Two cases are presented where administration of bromocriptine in the puerperium was associated with massive myocardial infarction. These incidents suggest the possibility that bromocriptine occasionally displays the vasoconstrictor effects of other (nonhydrogenated) ergot alkaloids. (AM J OBSTET GYNECOL 1986;155:371-2.)

Key words: Bromocriptine, myocardial infarction, puerperium, vasoconstrictor

Bromocriptine mesylate (Parlodel, Sandoz Pharmaceuticals), a dopamine receptor agonist, has been used widely for the suppression of lactation during the early puerperium (2.5 mg two or three times daily). Bromocriptine is a derivative of ergot, a powerful vasoconstrictor. It has been shown that hydrogenation of ergot alkaloids changes their pharmacologic effect from vasoconstriction to vasodilation. Accordingly, bromocriptine has been used even in conditions associated with vasospasm and in combination with vasopressor agents.

Inquiries involving catastrophic maternal outcome have brought two cases to our attention recently where massive myocardial infarction occurred post partum in patients receiving Parlodel.

Case reports

Case 1. Mrs. J. P. experienced her second pregnancy in 1981. She was 36 years old and weighed 79½ pounds. At 36 weeks' gestation, the systolic blood pressure increased by 20 mm Hg. A few days later she gave birth spontaneously to a 2035 gm baby. Postpartum uterine atony caused anemia (hemoglobin 8.3 gm, hematocrit 24.6%) requiring transfusion of two units of packed cells.

The patient was given ergonovine maleate tablets (0.2 mg every 4 hours for 24 hours) and Parlodel (2.5 mg tablets twice daily). When discharged on the fourth postpartum day, she continued taking Parlodel and ergonovine (0.2 mg three times daily, total of 12 tablets).

Three days later she developed severe headache followed by visual difficulties and "black spots" before the eyes. On the tenth postpartum day, the patient was rehospitalized. The blood pressure was 150/120 mm Hg, and the pulse rate 120 bpm. An electrocardiogram revealed sinus rhythm with periods of atrial-ventricular dissociation, premature ventricular contractions, and evidence of an acute anterolateral myocardial infarction.

The healing and scarring of the infarcted myocardium resulted in a left ventricular aneurysm. On discharge from the hospital her condition was consistent with a Class 4 New York State Heart Association status.

Case 2. Mrs. K. R. was 22 years old and pregnant for the second time in 1984. The low blood pressure level (51 to 58 mm Hg diastolic) increased significantly by term (148/85 mm Hg). Because of fetal compromise, she was delivered by cesarean section. Post partum, the patient received benquinamide hydrochloride (Emete-con, Roerig Pharmaceuticals) for 3 days (50 mg intramuscularly every 4 to 6 hours). She also received Parlodel (2.5 mg twice daily). The latter drug was continued after her return home 3 days post partum.

One day later, the patient fell ill. On the fifth postoperative day, she was found lifeless in her bathroom. Resuscitative efforts by the emergency squad and in the hospital failed. An autopsy revealed occlusion of the anterior descending branch of the left coronary artery by thrombotic emboli resulting in acute infarct of the myocardium and the corresponding portion of the intraventricular septum.

Comment

Of the pharmacologic agents that the patients received post partum, bromocriptine served as the common denominator. However, both patients received vasopressor agents along with bromocriptine.

Since ergonovine can cause myocardial infarction, it is noteworthy that it was administered in high doses to

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a very small woman. Thus the vasoconstriction and coronary occlusion may have resulted primarily from this drug. Nonetheless, the possibility that bromocriptine potentiated its effect deserves consideration.

In the second case, benzquinamide was discontinued 3 days before the cardiac accident. Thus the suspicion is high that the coronary occlusion resulted from bromocriptine alone.

It is tempting to speculate that in some individuals, as a result of a genetically determined error of metabolism, there is an inability to distinguish between hydrogenated and nonhydrogenated ergot alkaloids. Such persons may respond to the administration of either with vasoconstriction.

It is now recognized that the metabolisms of ergonovine, benzquinamide, and bromocriptine involve the P⁴⁵⁰ enzyme system. Therefore, a competitive inhibition can be postulated between the metabolisms of bromocriptine on the one hand and ergonovine and benzquinamide on the other. This may lead to activation of the α -adrenergic receptors resulting in generalized vasoconstriction. Another possible mechanism could be a change in the sensitivity of the dopamine receptors with similar result.

Including all suspected cases reported through the Food and Drug Administration¹ and those recently published,² about 20 observations suggest a possible vasopressor effect of bromocriptine. The cases presented here are the first ones where the outcome involved myocardial infarction. However, since the potential role of this drug remained unconsidered in both cases, it seems possible that bromocriptine-related complications have been underreported in the past.

In the Perinatal Center in Newark, New Jersey the postpartum use of bromocriptine was discontinued in January, 1984, after Case 1 had come to our attention. At the same time, we reported our suspicion to the Food and Drug Administration. In the light of other similar observations,^{1,2} we intend to continue this policy until more information about the side effects of bromocriptine becomes available and potential vascular complications can be predicted and thus prevented.

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Pregnancy in a patient with a history of myocardial infarction and coronary artery bypass grafting

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A 37-year-old woman conceived after experiencing a myocardial infarction and undergoing three-vessel aortocoronary artery bypass grafting. Pregnancy was complicated by angina, which was successfully treated with propranolol and bed rest. At term the patient underwent vaginal delivery, without evidence of intrapartum myocardial ischemia or failure. (*AM J OBSTET GYNECOL* 1986;155:372-3.)

Key words: Pregnancy, cardiac disease, myocardial infarction, ischemic heart disease, coronary artery bypass grafting

Ischemic heart disease is uncommon in obstetric practice. We report here a case in which a patient underwent pregnancy after myocardial infarction and aortocoronary artery bypass grafting.

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Case report

A 37-year-old white woman, gravida 1, para 0, was referred to the University of Iowa Hospitals and Clinics at 10 weeks' gestation. Twenty months earlier the patient had suffered an anterior myocardial infarction, documented by electrocardiographic and enzyme changes. Her risk factors for ischemic heart disease included 20 pack years of smoking, obesity, and a history of early death due to heart disease in both her mother and maternal grandmother; she had no history of hypertension, diabetes, or hyperlipidemia. Subse-

quent unstable angina prompted performance of three-vessel aortocoronary artery bypass grafting 7 months after myocardial infarction.

At referral the patient stated that she had experienced no angina in the 13 months since operation. Her only medication was digoxin. Her weight was 110 kg; there was no physical evidence of congestive heart failure. The arterial blood Po_2 was 82 mm Hg. Serum triglyceride and cholesterol levels were within normal limits. The electrocardiogram was consistent with an old anteroseptal infarct. An echocardiogram revealed left atrial enlargement, an akinetic septum, and reduced left ventricular systolic function. Nevertheless, our cardiology consultants recommended discontinuation of digoxin. The patient clearly stated her intention to continue with her pregnancy, and she declined genetic amniocentesis.

At 26 weeks' gestation the patient refused admission to our intermediate care inpatient facility. At 27 weeks' gestation she experienced three episodes of presumed angina, relieved by nitroglycerin. An exercise tolerance test was terminated at 1 minute of Stage II because of shortness of breath and 2.5 mm ST segment depression. She was begun on a regimen of propranolol, 40 mg three times daily, and was encouraged to remain at bed rest. At 28 weeks' gestation she had an abnormal glucose tolerance test, and she ultimately required eight units of daily subcutaneous insulin.

At 36 weeks' gestation the patient agreed to be hospitalized. Serial ultrasound examinations had documented appropriate fetal growth, and weekly nonstress tests were reactive. At 40 weeks' gestation the cervix was deemed favorable, and the patient underwent induction of labor by amniotomy (which revealed meconium-stained fluid) and administration of oxytocin. Cardiovascular precautions included (1) continuation of propranolol throughout labor, (2) maintenance of lateral uterine displacement, (3) administration of supplemental oxygen, (4) continuous monitoring of the maternal electrocardiogram, and (5) early institution of continuous infusion epidural analgesia, which was maintained until delivery.

Thirteen hours after amniotomy, the occurrence of repetitive late decelerations prompted fetal scalp blood sampling. All three pH values were >7.29 , and the decelerations resolved. Subsequently the patient had a temperature of 38.4°C . Ampicillin and gentamicin were begun for treatment of presumed chorioamnionitis. Eighteen hours after amniotomy the cervix was completely dilated. One hour later, moderate variable decelerations, followed by terminal bradycardia, prompted midforceps delivery of a 3230 gm infant in the occipitoposterior position. Apgar scores were 1 (the infant was intubated for suction of meconium) and 7. Analysis of umbilical cord blood suggested a modest respiratory acidosis (umbilical venous pH = 7.220, PCO_2 = 45.0 mm Hg, and base excess = -8.4 mmol/L; umbilical arterial pH = 7.082, PCO_2 = 63.4 mm Hg, and base excess = -11.1 mmol/L).

The placental culture grew *Escherichia coli*. Parenteral

antibiotics were discontinued after 48 hours. The patient was discharged on the fourth postpartum day, having had no symptoms of myocardial ischemia or failure during the intrapartum or postpartum periods. The infant was discharged on day 10 after a 10-day course of parenteral antibiotics.

Comment

Pregnancy has generally been discouraged in women with ischemic heart disease. However, Reece et al.¹ recently reported a case in which a diabetic woman conceived 2 years after undergoing single-vessel aortocoronary artery bypass grafting for treatment of unstable angina. "Deterioration of nephropathy and retinopathy" prompted successful delivery by elective cesarean section at 34 weeks' gestation.¹ As more women delay childbearing and as aortocoronary artery bypass grafting is performed more frequently, pregnancy after aortocoronary artery bypass grafting may occur more often. Mahar et al.² reported a low frequency of morbidity and mortality in patients who had undergone major noncardiac surgery after aortocoronary artery bypass grafting. They concluded that prior aortocoronary artery bypass grafting may have a protective effect for subsequent noncardiac surgery. It is not yet possible to extend their conclusions to pregnancy after aortocoronary artery bypass grafting.

We wish to emphasize four principles of management. First, cardiac function during pregnancy may be assessed by noninvasive means (that is, electrocardiogram, echocardiogram, exercise tolerance test). Second, propranolol reduces the frequency of anginal episodes by reducing myocardial oxygen demand; we believe that this advantage is of greater importance than the potential fetal-neonatal disadvantages previously reported. Once begun, propranolol should not be discontinued abruptly but should be continued intrapartum, lest a rebound increase in β -adrenergic receptor stimulation result in myocardial ischemia. Third, vaginal delivery is not contraindicated; we prefer to reserve cesarean section for obstetric indications. Finally, conduction anesthesia is useful in reducing myocardial stress during both the first and second stages of labor.

We gratefully acknowledge the assistance of Larry W. Richard, M.D., in the antenatal care of this patient.

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Hypothesis: Menstruation is a steroid-regulated, cyclic, autoimmune process

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There is presented an hypothesis that menstruation occurs when estrogen or progesterone withdrawal permits a previously hormone-blocked interaction between an endometrial autoantigen and its specific autoantibody, thereby inducing the characteristic necrosis and sloughing of the endometrium. Experimental and clinical studies are outlined to test this hypothesis. (AM J OBSTET GYNECOL 1986;155:374-5.)

Key words: Menstruation, autoimmune, steroid-regulated

This communication presents the hypothesis that normal menstruation is a steroid-regulated, autoimmune process. It is noteworthy that a major portion of the endometrium in primates undergoes periodic necrosis and sloughing associated with blood loss at a time in the ovarian cycle when the gonadal hormones reach their lowest titer.¹ Moreover, withdrawal of exogenous gonadal steroid, either estrogen or progestin or both, leads to "withdrawal bleeding." The nature of the supportive effect on the endometrium of either endogenous or exogenous gonadal steroids is not understood.

It is here proposed that just as the adrenal steroids serve to block more generally systemic antigen-antibody interaction in numerous tissue sites, the gonadal steroids have become highly specialized in their capacity to keep an endometrial antigen from interacting with a possible endometrial autogenous antibody, thus assuring the survival of the endometrium until the titer of gonadal steroid falls below the requisite plasma level. At that point it may be considered that the then uninhibited antigen-antibody interaction results in necrosis and rejection of the functional portions of the endometrium. By the same token, premenstrual edema would represent an initial phase of the impending autoimmune process.

Certain disorders of menstruation may be rationalized in accordance with this view. Thus secondary amenorrhea, which is not usually accompanied by atrophy of the vaginal epithelium even after many years' duration, would represent a failure of the formation of the immune complex because of the inhibitory effect

of a persistently low level of estrogen. Menorrhagia would represent an incomplete antigen-antibody interaction to a persistent but partially necrosed endometrium. Intermenstrual bleeding and spotting such as occurs during the use of oral contraceptive steroids would be construed as due to a quantitatively fluctuating titer of steroid but with a titer sufficient at times to permit antigen and antibody to interact.

This hypothesis is supported by several known clinical features of now recognized autoimmune phenomena, for example, the great preponderance of females among patients with autoimmune disease ranging from myasthenia gravis (2:11) to lupus erythematosus (9:1).² This suggests that the female organism is more extensively involved in autoimmune processes such as menstrual function and may consequently be more susceptible to their derangement.

This approach to the normal menstrual process is subject to experimental testing. For example, both in the clinic setting and in experimental primates it would be instructive to test the potential effects of such immunosuppressant drugs as Imuran or cyclosporine on spontaneous or steroid hormone-induced menstruation. Possibly, a careful review of the menstrual history of otherwise immunologically normal women exposed to these immunosuppressant agents would provide pertinent data.

That autoimmune processes may relate to normal physiologic functions in the absence of autoimmune disease has been suggested by Grabar.³ He suggests that both endogenous and exogenous substances that have been incompletely degraded by specific enzymatic systems may be capable of inducing the formation of autoantibodies.

The hypothesis that the normal cyclic sloughing of the endometrium with its associated tissue necrosis and hemorrhage is brought about by a steroid-controlled

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interaction of a normally occurring endometrial antigen and an antibody generated in the pubertal period merits clinical and experimental study.

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Thrombolytic therapy for pulmonary embolism in pregnancy: A case report

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A case of a 19-year-old woman whose pregnancy was complicated by hemodynamically significant pulmonary thromboemboli is reported. The diagnosis was confirmed by radionuclide ventilation-perfusion lung scanning, and urokinase thrombolytic therapy resulted in a good therapeutic response with minimal adverse effects. (*Am J Obstet Gynecol* 1986;155:375-6.)

Key words: Thrombolytic, urokinase, pregnancy, thromboembolism

We report a case of pulmonary thromboemboli in a pregnant woman. It is of interest because it is the first to describe the effective and safe use of urokinase thrombolytic therapy in this context.

Case report

A 19-year-old black woman, gravida 3, para 2, in her twenty-eighth week of pregnancy, was brought to the emergency center after having been found at home unresponsive. On the day before presentation, she had complained of 2 days of recurrent episodes of chest tightness, followed by dyspnea, nonproductive cough, and near syncope.

On arrival to the emergency center, she was noted to be lethargic, disoriented, and in respiratory distress. Vital signs were: blood pressure, 102/50 mm Hg; pulse, 148 bpm; respiratory rate, 30 breaths per minute; temperature, 98° F orally. Physical examination was remarkable for the heart, which revealed a tachycardia, accentuated second heart sound (S₂), systolic ejection flow murmur, and right ventricular heave; the lungs, which were clear to auscultation; and the abdomen,

which revealed a 28 weeks' size gravid uterus with fetal heart tones and a negative stool occult blood test. The lower extremities exhibited only trace edema and no evidence of thrombophlebitis. Aside from lethargy and disorientation, the neurological examination was unremarkable.

Arterial blood pH and gas analysis on room air revealed a pH of 7.47, Pco₂ of 25.5 mm Hg, and a Po₂ of 54 mm Hg, with a calculated oxygen saturation of 92%. Prothrombin and activated partial thromboplastin times were normal at 10 and 27 seconds, respectively. Serum electrolytes were normal except for a serum bicarbonate of 18 mEq/L. An electrocardiogram revealed only a sinus tachycardia, and a chest roentgenogram with abdominal shielding was interpreted as normal. Abdominal ultrasonography showed a single breech fetus with a normal fundal placenta and an estimated gestational age of 29.2 ± 1.5 weeks.

A diagnosis of pulmonary thromboembolism was made and the patient was admitted to the medical intensive care unit for further evaluation and care. An emergency radionuclide ventilation-perfusion lung scan with xenon 133 gas and technetium Tc-99m-human serum albumin microspheres revealed bilateral multiple segmental perfusion defects with normal ventilation and was interpreted as highly probable for multiple pulmonary emboli.

Because of the patient's tenuous hemodynamic status, it was decided to initiate thrombolytic therapy with intravenous urokinase. An initial intravenous loading

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dose of 4400 IU/kg during 10 minutes followed by a constant infusion of 4400 IU/kg/hr for 12 hours was administered. Thrombolysis 4 hours into therapy was noted to be increased by a partial thromboplastin time of 36 seconds and fibrin split products titer >1:40. A simultaneous sample for determination of thrombin time was lost.

During the ensuing 12 hours of therapy, marked symptomatic improvement manifested by clearing of mental status and resolution of dyspnea was noted. Vital signs improved, and on repeat cardiac examination the intensity of S_2 was decreased and both the flow murmur and right ventricular heave were absent. The only adverse effect noted was an episode of mild, self-limited macroscopic hematuria.

After the urokinase infusion was discontinued, a continuous intravenous heparin infusion was begun and adjusted to maintain a partial thromboplastin time at one and a half to two times the control value. After 5 days of continuous heparin, subcutaneous heparin was administered at full anticoagulant doses. She was discharged on the eleventh hospital day on a regimen of heparin, 10,000 IU subcutaneously every 6 hours, and ferrous sulfate.

Aside from two brief hospital readmissions for adjustment of anticoagulant therapy, she did well. Two months after the initial admission, she had an uncomplicated vaginal delivery of a healthy term infant.

Comment

Pulmonary thromboembolism is a recognized cause of morbidity and mortality during pregnancy. The risk of documented pulmonary emboli during pregnancy was found to be at least 0.09 per 1000 pregnancies and mortality has been estimated to be 12.8% for untreated cases and 0.7% for treated cases.¹ Although thrombolytic therapy has proved effective for occlusive proximal deep vein thrombosis and for massive and submassive pulmonary thromboembolism, experience in pregnant patients has been limited. In our review of the literature, we found no cases describing the use of urokinase in pulmonary embolism during pregnancy and only one series reporting the use of streptokinase in deep venous thrombosis (some with pulmonary embolism) during pregnancy.²

Moreover, reports concerning the various diagnostic

modalities used in thromboembolic disease in pregnancy are also sparse. In general, the degree of fetal risk from irradiation with either pulmonary arteriography or lung scanning has been unknown. Recently, it has been shown that fetal radiation exposure during radionuclide ventilation-perfusion lung scanning is insignificant compared with the risk to mother and fetus from undiagnosed and untreated pulmonary embolism.¹

Our patient presented in the twenty-eighth week of pregnancy with clinical evidence of acute pulmonary embolism and hemodynamic compromise. The diagnosis was confirmed by a high-probability lung scan and the benefits of thrombolytic therapy were felt to outweigh the risks to mother and fetus. Urokinase was selected over streptokinase because (1) it is a human protein and theoretically less antigenic; (2) a shorter duration of total infusion time is recommended; and (3) febrile reactions to infusion are not as frequent.³

Therapeutic benefit was confirmed in this case by prompt vital sign and clinical improvement. Monitoring of thrombolytic therapy ideally includes determination of the thrombin time 4 hours into therapy to confirm activation of the fibrinolytic system. The partial thromboplastin time and fibrin split products titer can also be used but in general are not as sensitive as the thrombin time.³ Adverse effects, which are predominantly bleeding and rarely allergic reactions, were limited to hematuria that did not necessitate discontinuation of thrombolytic therapy.

This case provides evidence for the efficacy and relative safety of urokinase thrombolytic therapy in the treatment of hemodynamically significant pulmonary embolism in pregnancy. Proof of absolute safety requires more experience.

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Diagnosis and management of fetal facial clefts

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Fetal facial clefts were identified sonographically in 12 cases, 10 (83%) of which had other structural anomalies. When a facial cleft is diagnosed prenatally a careful search of the fetus for associated anomalies is indicated. With the finding of an autosomal trisomy in four of the 10 cases (40%) with associated anomalies, cytogenetic amniocentesis is desirable to aid in diagnosis and subsequent obstetric management of these patients. (AM J OBSTET GYNECOL 1986;155:377-9.)

Key words: Ultrasound, congenital anomalies, facial clefts

The recent improvement in the resolution of real-time ultrasound equipment and more operator experience now permit the accurate antenatal detection of fetal facial anomalies.¹⁻³ The present report focuses on a series of 12 cases in which the diagnosis of a facial cleft was made antenatally as early as 16 weeks. The high rate of abnormal karyotypes with multiple congenital abnormalities in these fetuses is discussed as are the implications regarding obstetric management.

Methods

Twelve fetuses with cleft lip and palate were identified in an obstetric ultrasound laboratory where the fetal face is routinely imaged. The sonograms were performed with an Acuson 128 or ATL-MK 300 real-time system with a 3.5 and a 3 mHz transducer, respectively. Views of the fetal face include coronal sections through the soft tissues of the face as well as sagittal cuts showing the face in profile. The abnormal sonograms prompted cytogenetic studies in four patients at the discretion of the individual attending obstetrician.

Results

Twelve fetuses (Table I) had the prenatal sonographic diagnosis of cleft lip and palate, and 10 (83%) of these had additional congenital anomalies also recognized. Of these 12 cases, four (33%) fetuses had a final diagnosis of an autosomal trisomy. If this observation is limited only to fetuses with anomalies in addition to cleft lip and palate the incidence of autosomal

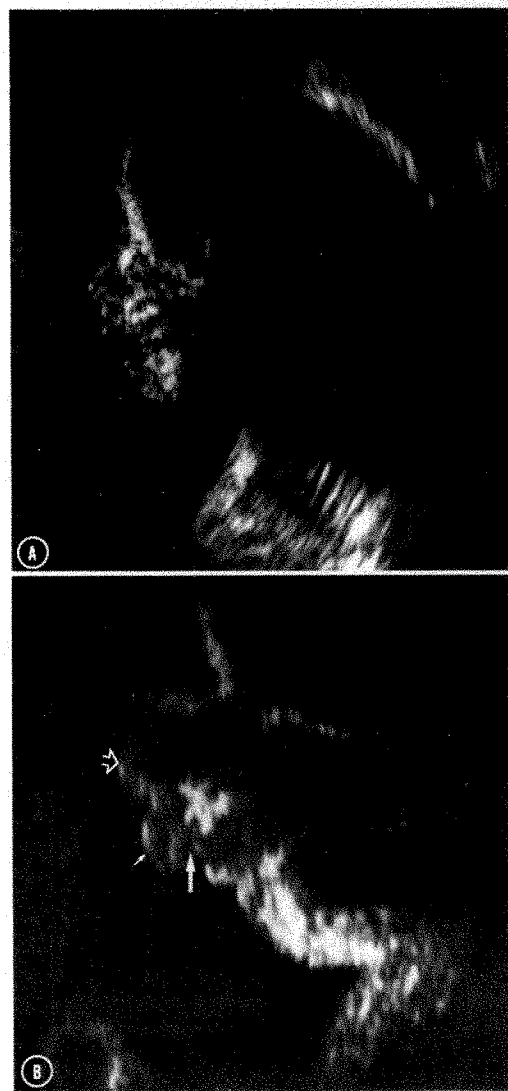


Fig. 1. A. Sagittal view of normal fetal profile at 23 weeks. B. Sagittal view of the lower face of fetus with central cleft (long solid arrow). The abnormal upper lip is indicated by the small solid arrow and the nose is indicated by the open arrow (Case No. 5).

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Table I. Case histories

<i>Case No.</i>	<i>Age (yr)</i>	<i>Gestational age at diagnosis (wk)</i>	<i>Genetic amniocentesis</i>	<i>Ultrasound findings</i>	<i>Outcome and follow-up</i>
1	26	19	Not done	Cleft lip and palate, hydrocephalus	Pregnancy terminated at 20 wk. Trisomy 13. Ultrasound findings confirmed
2	31	23	Yes, normal chromosomes	Hydrocephalus and large facial cleft	Premature labor at 28 wk. Ultrasound findings confirmed. Neonatal death. Dysmorphic brain
3	23	35	Yes, normal chromosomes	Cleft lip and palate, omphalocele, left clubfoot	Delivery of stillborn infant at term. Ultrasound findings confirmed
4	32	36	Not done	Cleft lip and palate	Uncomplicated term delivery. Unilateral cleft lip and palate confirmed as only abnormality
5	29	38	Not done	Abnormal face with central cleft, abnormal intracranial contents with mass polyhydramnios	Normal chromosomes. Orofacial digital syndrome. Primary cesarean section for cephalopelvic disproportion. Neonatal death
6	31	19	Not done	Cleft lip and palate, holoprosencephaly	Pregnancy terminated at 19 wk. Trisomy 13. Neonatal death
7	31	38	Not done	Cleft lip and palate, hypoplastic left heart, intrauterine growth retardation	Ultrasound findings confirmed. Neonatal death due to hypoplastic left heart
8	31	37	Not done	Cleft lip and palate, holoprosencephaly	Ultrasound findings confirmed. Trisomy 13. Neonatal death
9	20	26	Not done	Cleft lip and palate holoprosencephaly	Ultrasound findings confirmed. Neonatal death
10	30	31	Yes, normal chromosomes	Cleft lip and palate, ventricular septal defect, cystic hygroma	Ultrasound findings confirmed. Neonatal death
11	24	18	No	Cleft lip and palate	Pregnancy terminated at 18 wk. Ultrasound findings confirmed
12	21	31	Yes	Cleft lip and palate, polyhydramnios, intrauterine growth retardation, ventricular septal defect	Ultrasound findings confirmed. Trisomy 18. Premature labor at 34 wk. Vaginal delivery. Neonatal death

trisomies increases to 40% (four of 10). There were three cases of trisomy 13 (Cases 1, 6, and 8) and one case of trisomy 18 (Case 12). In all four cases of autosomal trisomy other congenital anomalies were recognized antenatally. In the three cases where the diagnosis of cleft palate was made before 20 weeks the pregnancy was electively terminated (Cases 1, 6, and 11). In the nine pregnancies that were electively continued (Cases 2, 3, 4, 5, 7, 8, 9, 10, and 12) one infant was stillborn (Case 3) and seven did not survive the neonatal period, for a perinatal mortality rate of 89%. The one infant who survived (Case 4) had no other associated congenital anomalies. In no instance was an autosomal trisomy diagnosed before ultrasound ex-

amination. Representative sonograms from our cases are shown in Figs. 1, A and B, 2, and 3.

Comment

By 35 days of uterine age, complete closure of the upper lip should have been effected and failure of this closure may impair further closure of the palate (normally occurring in the eighth or ninth week), and thus cleft lip and palate result. The risk of recurrent cleft lip and palate within a family can be between 3% and 10%. Although the most common form of cleft lip and palate is a primary defect without other congenital anomalies, cleft lip and palate figure as part of many different congenital syndromes that are multifocal and

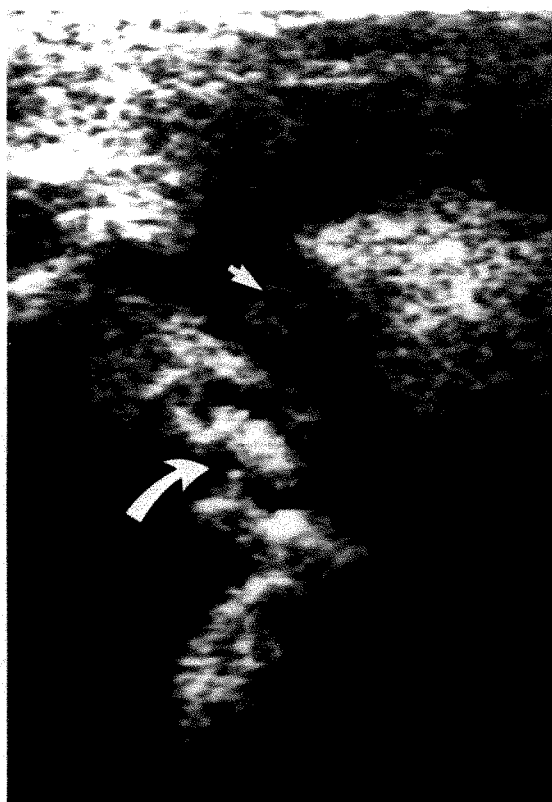


Fig. 2. Coronal view of fetal face at term showing the cleft lip (curved arrow). The right eye is indicated by the straight arrow (Case No. 7).

can be chromosomal as well.⁶ Associated congenital abnormalities occur with a frequency of 2% to 50% of cases, depending on the type of facial cleft subtype.⁷ The high rate of other congenital abnormalities in our patients (83%) helps to emphasize the importance of a careful structural survey of the fetus once a facial cleft is recognized. The incidence of clefting is 60% and 40%, respectively, in fetuses with trisomy 13 and 18.

Our finding of trisomy 13 in three cases and trisomy 18 in one case (40%) indicates that cytogenetic amniocentesis may have a high yield in cases where a facial cleft with other anomalies is diagnosed antenatally. As we currently perform amniocentesis for patients whose risk for chromosomal abnormality is <1%, for instance, for advanced maternal age or low maternal serum α -fetoprotein level, we feel, on the basis of this preliminary observation, that an amniocentesis should be considered to aid in obstetric management when a facial cleft with associated anomalies is visualized ultrasonographically. Although we are unable to draw definite conclusions from our nine cases in which the pregnancy was not terminated, it is of note that in eight cases the fetus did not survive the perinatal period. The only survivor had no other recognizable abnormalities. More work is being done at present in our laboratory to de-



Fig. 3. Magnified coronal view of large facial cleft at 18 weeks (Case No. 11). Bony cleft is indicated by solid arrows. Nasal bones are shown by the open arrows.

termine whether the type of clefting present in a fetus can be accurately seen in order to predict which fetuses have simple clefts and which are likely to have other congenital abnormalities.

In conclusion, we stress the importance of fetal facial examination during prenatal ultrasound studies and a thorough search for other abnormalities when a cleft is recognized. Consideration should also be given to performing cytogenetic amniocentesis, as there was a high yield of abnormal karyotypes in this select group of patients. Knowledge of an abnormal karyotype is especially useful in aiding obstetric management, particularly now that the diagnosis of facial clefting is made reliably before 20 weeks' gestation.

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Sclerema neonatorum and joint contractures at birth as a potential complication of chronic in utero hypoxia

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Three term neonates presented with an unusual combination of skin changes (sclerema) and joint contractures at birth. In each case a prolonged period of fetal hyporeactivity was reported by the mother. Each child demonstrated a number of clinical findings consistent with chronic fetal hypoxia. Each child recovered with normal joint function. We postulate that sclerema neonatorum and joint contractures are another, though poorly recognized sequela of chronic in utero hypoxia and are preceded by a prolonged period of diminished fetal activity. (AM J OBSTET GYNECOL 1986;155:380-1.)

Key words: Sclerema neonatorum, joint contractures, hypoxia

During a 12-month period three neonates (35 to 38 weeks' gestation) presented at birth with an unusual association of sclerema neonatorum and reversible joint contractures. Each pregnancy was complicated by pregnancy-induced hypertension, a poorly reactive non-stress test, and fetal distress by heart rate monitoring necessitating cesarean section delivery. Each mother reported a variable period of decreased fetal movements lasting 2 to 4 weeks before delivery. Fetal hyporeactivity was confirmed at the time of hospital admission. Each mother was delivered at a community hospital and was then transferred to a local neonatal intensive care facility (The Johns Hopkins Hospital or Francis Scott Key Medical Center). Each of the neonates presented with intrauterine growth retardation and profound birth hypoxia (5-minute Apgar score <5) and demonstrated a neonatal course punctuated by multisystem organ dysfunction consistent with chronic prenatal hypoxia. One such representative case is presented here.

Case report

A 2480 gm black male infant was born at 38 weeks to a 20-year-old primiparous woman with type A-positive blood. She was not in labor but was admitted to the hospital for control of hypertensive preeclampsia and required therapy with magnesium sulfate and hydralazine hydrochloride. A history of decreasing fetal movements during the previous 2 weeks was reported by the mother at the time of her admission. Spontaneous late decelerations with fetal bradycardia during external monitoring led to an emergency cesarean sec-

tion with the mother under epidural anesthesia. Somewhat diminished amniotic fluid, light meconium staining, and a nuchal cord were noted. An Apgar score of 1 required immediate cardiac massage and bag and mask ventilation and intubation at birth. Vigorous resuscitation raised the 5-minute Apgar score to 3 and his first spontaneous movements were recorded at 20 minutes of age. In the nursery he was placed on a ventilator at 100% oxygen. Venous blood gas values at 20 minutes of age were: pH, 7.34; PO_2 , 54 torr; PCO_2 , 76 torr; initial dextrostix was 0 mg/100 ml. The initial hematocrit was 38%; the prothrombin time, partial prothrombin time, and fibrinogen were normal but the platelet count was low at 108,000/mm³. He was transported at 6 hours of age to Francis Scott Key Medical Center.

Admission temperature was 36.5° C, heart rate 140 bpm, respiratory rate 80 breaths per minute with the ventilator, and blood pressure 70/46 mm Hg. Weight was 2480 gm (<twenty-fifth percentile), length 48 cm (fiftieth percentile), and head circumference 33.25 cm (fiftieth percentile). Most remarkable was the child's brawny edema (sclerema), most noticeable in the trunk and extremities. No dependent edema was present in the back or scrotum and the face and head were spared. He was poorly reactive without spontaneous movement of the extremities. There were flexion deformities at the elbows, knees, and hips. Chest expansion was difficult because of sclerema of the chest wall. Breath sounds were diminished on the right, and heart sounds seemed distant. The abdomen demonstrated thick brawny edema and the intra-abdominal structures were difficult to palpate. The liver appeared enlarged to percussion. At 2 hours of age he began to demonstrate tonic-clonic seizures of all four extremities and was treated with phenobarbital.

An initial chest x-ray film demonstrated a pleural effusion on the right with mild cardiomegaly. Thirty-five milliliters of thin straw-colored fluid was aspirated from the right pleural space. Bacterial and viral cultures of the blood, urine, and pleural fluid were sub-

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sequently negative. A complete blood count revealed a hematocrit of 40% and a white blood cell count of $19,000/\text{mm}^3$ with 56 polymorphonuclear cells and 36 lymphocytes. The platelet count had fallen to $32,000/\text{mm}^3$. A transfusion of concentrated platelets and packed red blood cells was undertaken. The initial urea nitrogen was 24 mg/dl and creatinine 1.8 mg/dl.

Respiratory status improved rapidly during the first 24 hours although he continued to demonstrate cardiomegaly with a systolic ejection murmur consistent with tricuspid insufficiency. Oliguric renal failure ($<1 \text{ ml/kg/hr}$) continued for the first 36 hours of life. A sonogram of the abdomen defined a normal liver, spleen, and kidneys. An echocardiogram demonstrated a dilated right ventricle and right atrium with normal left ventricular function and a small pericardial effusion. A sonogram of the brain on the second day of life demonstrated no hydrocephalus or intracerebral bleeding, although slit-like ventricles consistent with cerebral edema were apparent.

The child's activity slowly improved with resolution of sclerema during the first 10 days of life. His baseline weight was estimated to be 2000 gm. Muscle mass and strength in both upper and lower extremities appeared diminished. No further seizure activity was noted. He was extubated on day 4 and was breathing room air by day 5. Other problems noted during his hospitalization included: hypokalemia, hyponatremia, transient diabetes insipidus, persistent thrombocytopenia requiring two further platelet transfusions, and hyperbilirubinemia. The range of motion in his contracted joints improved consistently with physical therapy, and surgical intervention or casting was not required.

Comment

Sclerema is a "diffuse rapidly spreading, non-edematous, tallow-like hardening of the subcutaneous tissue,"¹ most usually described as a postnatal, frequently rapidly fatal consequence of hypothermia or gram-negative sepsis. Sclerema is distinguishable from the pitting edema of anasarca or hydrops fetalis, which is most prominent in the dependent regions of the body and prominently involves the face and head. Although

chronic hypoxia is not commonly included as a pathogenic mechanism of sclerema, in the review of Hughes and Hammond¹ three of 19 cases occurred at birth and two cases were seen in infants of preeclamptic mothers. Since previous reports have not focused on sclerema occurring at birth, associated joint contractures have not been noted. Although a mild degree of oligohydramnios was noted in two of our three prenatal ultrasound examinations, in no case was this severe enough to lead independently to arthrogryposis. Likewise, none of our three neonates demonstrated pulmonary hypoplasia. It is interesting to speculate that both defects (sclerema and joint contractures) might be related to redistribution of blood flow away from the fetal carcass (skin, muscle, bone), a physiologic adaptation to hypoxia seen in fetal² as well as postnatal life. A prolonged period of placental insufficiency in the hypoxic fetus could then lead to diminished body movements followed by ischemic changes of the skin (sclerema) and resultant joint contractures. This theory is supported by the postnatal evidence of chronic asphyxial disease in our patients—central nervous system, lung, liver, heart, hematopoietic, and renal organ involvement.

In summary, this report reemphasizes the association of decreased fetal body movements, diminished amniotic fluid volume, and poor heart rate reactivity as an indicator of uteroplacental insufficiency and demonstrates the multiorgan system involvement this process may impart to the newborn infant. Additionally, fetal hypoactivity and ischemic skin blood flow may explain both sclerema and joint contractures when hypothermia and sepsis are not etiologic possibilities.

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Condition and performance of the perfused human placental cotyledon

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The perfused human placental cotyledon was examined with respect to its viability, metabolic state, and performance. During the ischemic period before the start of perfusion, tissue adenosine triphosphate concentration and other measures of energy state fell rapidly to about half of the estimated in vivo value. During the subsequent perfusion, energy levels remained relatively stable but did not recover appreciably toward in vivo values. A very low transplacental leakage of inulin and a small cellular potassium loss indicate relative intactness of membrane function, but there were differences from the in vivo state in levels and balance of metabolic regulators adenosine triphosphate, adenosine monophosphate, and adenosine triphosphate/adenosine monophosphate ratio, and a more reduced cytoplasmic reduced nicotinamide adenine dinucleotide/ionized nicotinamide adenine dinucleotide couple. However, rates of oxygen and glucose consumption and lactate production and the maintenance of physiologic upward maternal-to-fetal concentration gradients of amino acids lead us to conclude that despite differences in energy and metabolic states the perfused cotyledon remains substantially intact and functions in certain respects comparably with the in vivo state. (AM J OBSTET GYNECOL 1986;155:382-8.)

Key words: Viability of perfused human placenta; performance of perfused human placenta; placental energy state; human placental metabolism; human placental transfer; placental membrane function

The human placental cotyledon perfused in vitro is becoming an accepted model for the study of placental function, but the usefulness of results from it depends on an accurate and critical assessment of the degree to which it remains viable and also on whether it performs in vitro similarly or at least comparably to the way it functions in vivo.

The most acceptable tests of viability are changes that occur early during the onset of any deterioration of condition, that are, if possible, part of the cause of tissue injury, and that are sensitive, that is, large enough to be readily measured. Some of the earliest measurable changes in the development of tissue injury, for example, during hypoxia and ischemia, are an impairment of adenosine triphosphate (ATP) synthesis, leading to a decline in energy state, and disturbed membrane function, including the loss of intracellular potassium and a general membrane leakiness.

Measurement of energy state is particularly useful because an adequate metabolic energy supply is nec-

essary for the maintenance of normal tissue structure and function. However, for a proper assessment of the energy state of the perfused placenta it is necessary to find how it compares with that in vivo. Several reports of measurements of ATP, adenine nucleotides, or "energy charge" in the perfused human placenta have appeared,¹⁻³ but there has been no attempt to assess to what extent they reflect in vivo values.

We have therefore set out to investigate not only the energy state of the cotyledon during perfusion but also how it compares with estimates of energy levels in vivo and how these change during the ischemic period before perfusion starts. In addition to this we have looked at measures of membrane integrity and tissue oxidation-reduction state, the latter because it also changes rapidly and markedly during hypoxia and ischemia.

To measure placental performance we have investigated whether the perfused cotyledon can maintain the upward maternal-to-fetal concentration gradients of amino acids that have been found in vivo, as this is an important placental function that requires substantial amounts of metabolic energy as well as adequate membrane integrity.

Most studies have included measurements of rates of oxygen and glucose consumption and lactate synthesis¹⁻³ as examples of placental metabolism (although in vivo rates in the human are not known) and for comparison we have done the same.

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Methods

Patients. Placentas were obtained from mothers undergoing elective cesarean section for reasons not indicating any placental abnormality, such as for previous cesarean section, breech presentation, and cephalopelvic disproportion. Cesarean sections were carried out at 38 to 39 weeks of gestation (estimated by ultrasound scan at 14 to 16 weeks), except for four at 40 weeks and one at 41 weeks. All were uncomplicated and had normal outcomes. Of the 68 cesarean sections, 61 were performed with the patient under epidural blockade with either 0.5% (w/v) bupivacaine hydrochloride (Marcaine, Duncan Flockhart, London, England) or 1.5% lignocaine hydrochloride (Xylocaine, Astra Chemicals, Watford, United Kingdom). The remaining seven cesarean sections were performed with the patients under general anesthesia with a mixture containing 0.9% alphaxalone and 0.3% alphadolone acetate (Althesin, Glaxo Laboratories, Greenford, Middlesex, United Kingdom) and nitrous oxide-oxygen. The mothers' ages averaged 32.3 ± 0.6 (SEM) years ($n = 68$) and 60 of them were multiparous. The birth weights of the babies delivered were 3.31 ± 0.05 kg (33 were male) and the placentas weighed 580 ± 12 gm.

Perfusion method. Placentas were taken only from cesarean sections because their condition, judged by energy state and tissue potassium concentration, was significantly better than those taken from vaginal deliveries.⁹ In each case, an undamaged cotyledon was perfused by the method of Schneider, et al.¹⁰ but with the fetal side positioned uppermost, as carried out by Penfold et al.² The chorionic artery was cannulated first and perfused slowly with oxygenated fetal medium ("preperfusion") while the rest of the procedure was completed. When the maternal perfusion was established the fetal preperfusion was switched to fetal perfusion proper and this moment was designated zero time. The fetal medium, in a volume of 350 ml, was recirculated and the maternal side was perfused as an open circuit. Preperfusion was started at 8.2 ± 0.4 minutes, and zero time was at 16.8 ± 0.6 minutes, after delivery. The maternal and fetal pressures, measured in the cannulas near the tissue, were initially adjusted to 60 to 80 and 40 to 60 mm Hg, respectively. For the 11 perfusions that were run for 60 minutes, the actual pressures at 20, 40, and 60 minutes were: maternal, 72.9 ± 3.8 , 83.4 ± 4.7 , and 84.1 ± 5.8 mm Hg; fetal, 51.3 ± 3.5 , 58.8 ± 4.0 , and 56.7 ± 3.8 mm Hg. Flow rates at 20, 40, and 60 minutes for the set of 60-minute perfusions whose cotyledon weights were measured were: maternal, 0.81 ± 0.10 (five perfusions), 0.73 ± 0.09 , and 0.73 ± 0.09 ml/min/gm; fetal, 0.23 ± 0.09 (five perfusions), 0.21 ± 0.09 , and 0.21 ± 0.09 ml/min/gm.

The maternal and fetal perfusion media were Krebs-

Ringer bicarbonate buffer containing 30 gm/L of bovine serum albumin (fraction V reagent grade, Miles Laboratories, Stoke Poges, Slough, United Kingdom), 5.5 mmol/L D-glucose, and the amino acids (A grade, Calbiochem-Behring, CP Laboratories, Bishops Cleeve, United Kingdom, or Sigma Chemical Co., Poole, United Kingdom) at physiologic concentrations. The maternal perfusate and fetal preperfusate were equilibrated with a 95% oxygen, 5% carbon dioxide (v/v) gas mixture, and the fetal perfusate was equilibrated with 4% oxygen, 5% carbon dioxide, and 91% nitrogen.

Assays. Unperfused placentas were freeze-clamped, with tongs cooled in liquid nitrogen, at different times after delivery by cesarean section. Zero time samples were clamped within 20 to 45 (38.8 ± 2.1 , SEM) seconds of removal. The frozen samples were stored in liquid nitrogen for assay. Either perfused cotyledons were freeze-clamped at the end of perfusion and stored in the same way, or the blanch area was excised and weighed. Perfusion medium samples were stored at -20°C .

Placenta and perfusion medium samples were deproteinized with perchloric acid, and portions of the supernatants were neutralized as described previously.⁹ ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), glucose, lactate, and pyruvate in the neutralized extracts were measured spectrophotometrically or fluorometrically by enzymatic methods, and potassium in unneutralized perchloric acid supernatants was measured by flame photometry as before.⁹ The amino acids tyrosine, phenylalanine, and tryptophan were assayed in the neutralized perfusion medium extracts by the fluorometric methods of Waalkes and Udenfriend,¹¹ McCaman and Robins,¹² and Bloxam and Warren,¹³ respectively; cysteine and cystine, by the spectrophotometric method of States and Segal¹⁴; and alanine and lysine, by the enzymatic methods of Williamson¹⁵ and Tiller and Bloxam.¹⁶ Perfusate oxygen concentrations were measured directly, immediately after sampling, with a Radiometer ABL2 blood gas analyzer. Inulin labeled with carbon 14 was determined in 1 ml aliquots of perfusate with an LKB 1215 Rackbeta liquid scintillation spectrometer.

Tissue uptake and output of metabolites on each side were calculated from the arteriovenous differences in concentrations (and perfusion flow rates determined by pump calibration).

Results

Placental adenine nucleotides. Fig. 1 shows the changes in the concentrations of the adenine nucleotides in placental tissue during the period between delivery and the start of perfusion and during 60 minutes of perfusion.

The total adenine nucleotide concentrations

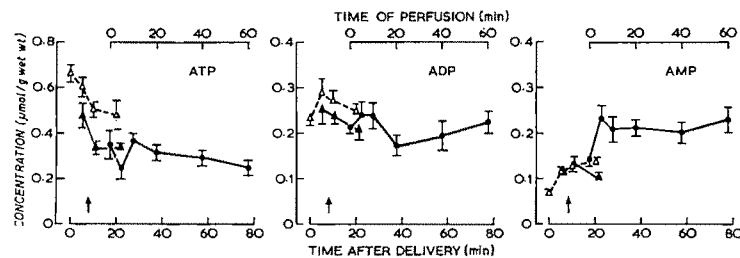


Fig. 1. ATP, ADP, and AMP concentrations in placental tissue before and during perfusion. Placentas were freeze-clamped at different times after delivery or after cotyledon perfusion (see Methods). Δ , Unperfused unwashed cotyledons, containing blood (each point, eight placentas); $\blacktriangle$, unperfused cotyledons rapidly washed through with ice-cold 0.15 mol/L sodium chloride on both maternal and fetal sides to remove blood before freeze-clamping (four, seven, and 11 placentas at 5, 10, and 20 minutes); $\bullet$, perfused cotyledons (five to seven placentas). All placentas were delivered by cesarean section. Bars indicate SEM and arrows indicate the time when preperfusion started. Changes of ATP and AMP concentrations in unperfused tissues were significant ($p < 0.05$ and $p < 0.001$, analysis of variance). For initial increase in AMP concentration during perfusion, $p < 0.05$. "In vivo" values were estimated by extrapolating data from unperfused placentas to the time of separation from the uterus (1 to 3 minutes before zero time). The washed cotyledons are the proper controls for the perfusions, but as there are no zero time values for these (because of the time taken for washing) results for unwashed cotyledons are also included. Values for washed cotyledons may thus be extrapolated parallel with values for unwashed cotyledons because the adenine nucleotide concentrations in placental blood remained essentially constant during the course of the experiment.

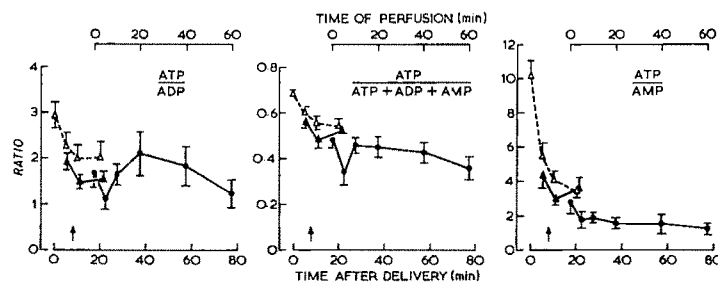


Fig. 2. Adenine nucleotide ratios in placental tissue before and during perfusion. Δ , Unperfused unwashed cotyledons, containing blood; $\blacktriangle$, unperfused cotyledons washed to remove maternal and fetal blood; $\bullet$, perfused cotyledons. For details see legend to Fig. 1. Changes in unperfused unwashed tissue were significant: ATP/AMP, $p < 0.001$; ATP/sum of nucleotides, $p < 0.005$; analysis of variance.

([ATP] + [ADP] + [AMP]) fell after delivery ($p = 0.05$, analysis of variance). Their values in placentas rapidly washed through to remove blood (see legend to Fig. 1) at 5, 10 and 20 minutes after delivery were 0.85 ± 0.08 (five experiments), 0.70 ± 0.04 (11 experiments), and 0.64 ± 0.04 (nine experiments) $\mu\text{mol/gm}$, respectively. However, there was no further change during the perfusions, when the values were as follows: at zero time of perfusion, 0.71 ± 0.05 ; at 20 minutes, 0.70 ± 0.03 ; at 40 minutes, 0.70 ± 0.06 ; and at 60 minutes, 0.70 ± 0.04 $\mu\text{mol/gm}$ (six experiments). Changes in the ratios of adenine nucleotide concentrations, ATP/ADP, ATP/(sum of adenine nucleotides), and ATP/AMP, in these experiments are shown in Fig. 2 (see Comment).

Inulin transfer. The appearance of ^{14}C -labeled inulin in the fetal perfusion medium, after addition to the

maternal medium, is shown in Fig. 3. Inulin clearance at 20, 40 and 60 minutes, respectively, was 0.71 ± 0.20 , 0.71 ± 0.24 , and $0.93 \pm 0.29 \times 10^{-3}$ ml/min/gm (five perfusions).

Tissue potassium loss. Fig. 3 also shows an apparent (although not statistically significant) fall of tissue potassium concentration during cotyledon perfusion, amounting overall to 19.8%.

Placental metabolism. Changes in the lactate/pyruvate concentration ratio (a measure of the redox state of cytoplasmic reduced nicotinamide adenine dinucleotide/ionized nicotinamide adenine dinucleotide) in placental tissue both before and during perfusion can be seen in Fig. 4 (see Comment). Rates of oxygen and glucose consumption and of lactate output by the perfused cotyledon are shown in Table I.

Transplacental amino acid gradients. It can be seen

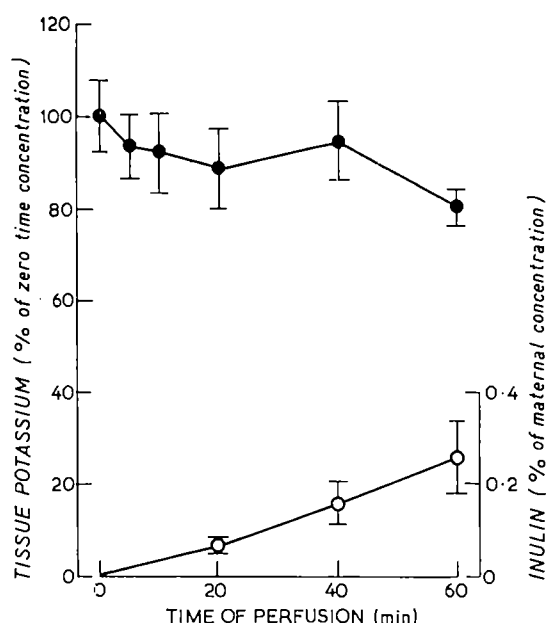


Fig. 3. Maternal-to-fetal inulin transfer (○) and tissue potassium loss (●) during cotyledon perfusion. ^{14}C -labeled inulin (500 mg/L) was present in the maternal medium throughout, and appearance in the fetal medium was measured (see Methods). Results are from five perfusions; cotyledon weights were 23.9 ± 0.7 gm. Potassium was measured in tissue of cotyledons after perfusion for the times shown (five to seven perfusions for each time). Zero time potassium concentration was 39.8 ± 3.1 (five perfusions) $\mu\text{mol/gm}$ of wet weight. Bars indicate SEM. The decrease of potassium concentration with time was not statistically significant (analysis of variance) but the 60-minute value was significantly lower than the zero time value when evaluated by Student's *t* test ($p < 0.05$).

from Fig. 5 that the upward maternal-to-fetal concentration gradients present at the start of perfusion were maintained by the placental cotyledon and steadily increased for tryptophan (after 10 minutes), alanine, and lysine. The small gradient of cysteine, which was initially not statistically significant, also increased during the course of perfusion ($p < 0.001$), and the measurements of cystine (which was not added to the perfusates) show that the cysteine changes were not complicated by oxidation to cystine (Fig. 5).

Comment

Energy state of the perfused cotyledon. The results show that during the ischemic period after separation from the uterus and before the start of perfusion there is a rapid and substantial decline in energy state of the placental cells. ATP concentrations fell to about half of the estimated *in vivo* value of 0.55 to 0.65 $\mu\text{mol/gm}$ (see legend to Fig. 1), with correction for ATP in the blood. ADP concentrations did not change significantly but those of AMP increased twofold over the value at delivery and perhaps fourfold over the estimated value of about 0.05 $\mu\text{mol/gm}$ at separation. When perfusion

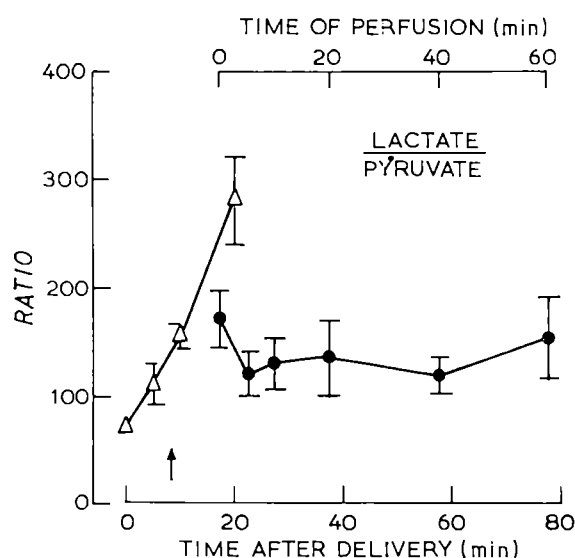


Fig. 4. Lactate/pyruvate concentration ratios in placental tissue before and during perfusion. Δ , Unperfused unwashed cotyledons; $\bullet$, perfused cotyledons. For details see legend to Fig. 1.

of the cotyledon was started there was an immediate further sharp, although transient fall of energy level (whether expressed as ATP, ATP/ADP, or ATP/total adenine nucleotides) presumably due to imbalance between the rates of recovery of ATP synthesis and utilization (see ATP/ADP values, Fig. 1). After this, the measures of tissue energy state were relatively stable but at values lower than those estimated to occur *in vivo*, and they tended to decline slowly, albeit not statistically significantly (Figs. 1 and 2), as perfusion progressed. However, AMP concentrations increased further during the first 5 minutes of perfusion, remaining stable thereafter at a level about threefold higher than at zero time of perfusion and possibly sixfold higher than at separation from the uterus (Fig. 1).

If the ATP/ADP ratio is a valid indication of the balance between ATP synthesis and utilization, the results (Fig. 2) suggest that oxidative phosphorylation recovered markedly up to 20 minutes of perfusion (ATP/ADP rising to 70% to 85% of the estimated *in vivo* value, Fig. 2) but declined, or did not keep pace with energy-utilizing reactions, thereafter.

The measurements of total adenine nucleotide concentration showed that there was some overall catabolism of the adenine nucleotides during the ischemia before perfusion, but once the perfusion started there was no further breakdown. The ratio ATP/sum of the adenine nucleotides (Fig. 2) indicates that *in vivo* approximately 70% of the tissue adenine nucleotides is present as ATP. At zero time of perfusion this proportion had dropped to about 50% (70% of the *in vivo* value) and at the end of perfusion it was 35% (half of

Table I. Rates of oxygen and glucose consumption and of lactate output by the perfused placental cotyledon

Rate ($\mu\text{mol}/\text{min}/\text{gm}$ wet weight)	Time of perfusion		
	20 min	40 min	60 min
Oxygen consumption	0.42 ± 0.06	0.38 ± 0.05	0.38 ± 0.06
Glucose consumption	0.28 ± 0.08	0.20 ± 0.04	0.23 ± 0.05
Lactate output	0.43 ± 0.09	0.48 ± 0.05	0.40 ± 0.05

Values are means $\pm$ SEM from five perfusions. There were no significant changes in rates with time.

the *in vivo* value). "Energy charge" $([\text{ATP}] + \frac{1}{2}[\text{ADP}])/([\text{ATP}] + [\text{ADP}] + [\text{AMP}])$, which has been used by several groups to assess the perfused placental cotyledon, fell by only 18% during the same period. However, "energy charge" is unlikely to be a sensitive criterion of placental viability because, as well as being a measure of potential rather than actual energy state, it is known to be extremely insensitive to conditions that may bring about large changes in the concentrations of ATP (or the other individual nucleotides).

Penfold et al.² measured adenine nucleotides in the perfused human placental cotyledon and found slightly higher values than ours for both ATP and ADP concentrations; ATP fell from $0.46 \mu\text{mol}/\text{gm}$ (mean of two perfusions) at 50 minutes to $0.21 \mu\text{mol}/\text{gm}$ (mean of two) at 2 hours, but ADP values were 0.41 and $0.29 \mu\text{mol}/\text{gm}$, giving values for ATP/ADP and ATP/sum of adenine nucleotide ratios that were similar to ours. Krantz et al.,¹ perfusing the whole placenta, found a tissue ATP concentration of $0.32 \mu\text{mol}/\text{gm}$ (82% of the zero time value) after 2 hours of perfusion, when perfusion medium glucose concentrations similar to ours were used, but they found higher ATP concentrations when higher glucose levels were employed. Young and Schneider,³ using a procedure involving perfusion of the maternal side only, found values after 60 minutes of $0.51 \pm 0.05 \mu\text{mol}/\text{gm}$ for ATP, $0.18 \pm 0.01 \mu\text{mol}/\text{gm}$ for ADP, and $0.08 \pm 0.01 \mu\text{mol}/\text{gm}$ for AMP (23 experiments), indicating a better tissue energy state in their preparation than in ours. Bearing in mind the rapid fall of energy level in placental tissue after separation from the uterus (Figs. 1 and 2), this may be explained by the quicker establishment of maternal perfusion in their singly perfused preparation. However, perfusion of both the maternal and fetal sides is obviously necessary when placental transfer is to be studied.

Membrane integrity. Our value for transplacental clearance of inulin, which does not readily cross intact, and viable cell membranes is about $0.8 \times 10^{-3} \text{ ml}/\text{min}/\text{gm}$ (see Results) compared with $5.9 \times 10^{-3} \text{ ml}/\text{min}/\text{gm}$ found by Brandes et al.⁷ using a similar preparation. This is low, and it was also relatively constant (Fig. 3), indicating no gross membrane damage or significant

progressive deterioration. Similarly the degree of retention of tissue potassium (Fig. 3) indicates that the sodium pump remained reasonably functional and the cell membranes were not unduly leaky. The ability of the perfused cotyledon to maintain physiologic amino acid gradients (Fig. 5) supports this view.

Metabolic state. In view of the importance in other tissues of the adenine nucleotides, and their balances, as metabolic regulators, the deviations of the perfused cotyledon ATP and AMP concentrations and their ratios from the estimated *in vivo* values suggest that the metabolic state of the cotyledon may be somewhat different from that *in vivo*. It should be mentioned, however, that the importance of these effectors as regulators of the rate of glycolysis in the human placenta has recently been questioned.⁹

The rise of the placental tissue lactate/pyruvate ratio after delivery (Fig. 4) indicates, as expected, a rapid reduction of the cytoplasmic reduced nicotinamide adenine dinucleotide/ionized nicotinamide adenine dinucleotide couple during the period of ischemia before perfusion started. This change was largely reversed when perfusion started, but the lactate/pyruvate ratio stabilized at a value that suggests that the cytoplasmic oxidoreduction state during perfusion is more reduced than *in vivo*.⁹ A similar finding has been reported by Young and Schneider.³ These altered patterns of both the adenine and nicotinamide nucleotides are changes that could occur with tissue hypoxia. However, the maternal venous perfusion medium still contained 18 kPa of oxygen (compared with oxygen tension of 13 kPa in arterial blood *in vivo*) and there was an accumulation of oxygen in the fetal medium, suggesting an adequate oxygen supply. It is therefore also possible that some other factor such as the different substrate or hormonal environment could be responsible for these differences from the results *in vivo*.

Placental metabolism. The rate of perfused cotyledon oxygen consumption of about $0.4 \mu\text{mol}/\text{min}/\text{gm}$ that we find compares with 0.43 ± 0.13 (five experiments) found by Schneider et al.⁶ and 0.30 ± 0.04 (six experiments) and 0.22 ± 0.03 (nine experiments) $\mu\text{mol}/\text{min}/\text{gm}$ by Hauguel et al.⁸ and Illsley et al.,¹ respectively. Brandes et al.,⁷ with no oxidizable substrate

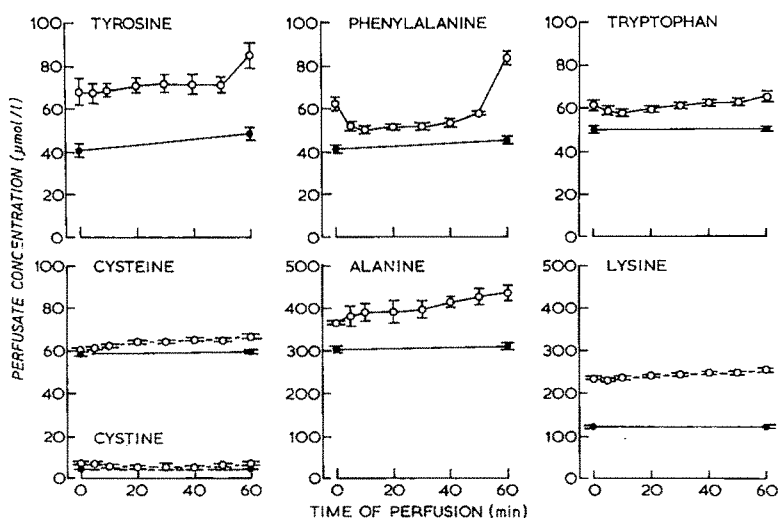


Fig. 5. Amino acid concentrations in the maternal and fetal perfusion media during cotyledon perfusion. ●, Maternal concentration; ○, fetal concentration. The maternal medium was perfused with one pass and the fetal medium was recirculated. Results are from six perfusions. Bars indicate SEM. Increases in fetal concentration during perfusion were significant for cysteine, lysine ($p < 0.001$), tryptophan (after 10 minutes), and alanine ($p < 0.05$, analysis of variance). The changes of phenylalanine concentration between 0 and 5 minutes and between 50 and 60 minutes were significant ($p < 0.02$ and $p < 0.001$, respectively, Student's t test).

added to the perfusion media, obtained a rate of $0.12 \mu\text{mol}/\text{min}/\text{gm}$. The rate of glucose consumption seems to depend on the concentration of available glucose.¹ Thus Schneider et al.⁶ and Hauguel et al.,⁸ using $8.3 \text{ mmol}/\text{L}$ glucose in the maternal medium, obtained rates of 0.50 ± 0.18 (five experiments) and 0.43 ± 0.07 (six experiments) $\mu\text{mol}/\text{min}/\text{gm}$, and we and Illsley et al.,¹ using $5.5 \text{ mmol}/\text{L}$ glucose, found rates of about 0.2 (Table I) and $0.16 \mu\text{mol}/\text{min}/\text{gm}$. Similarly, lactate production rates of 1.12 ± 0.12 (five experiments) and about $1 \mu\text{mol}/\text{min}/\text{gm}$ were found by Schneider's group⁶ and Young and Schneider³ (also using $8.3 \text{ mmol}/\text{L}$ glucose), whereas we and Illsley et al.¹ had rates of about 0.4 (Table I) and 0.18 ± 0.01 (nine experiments) $\mu\text{mol}/\text{min}/\text{gm}$, respectively. Bearing in mind the differing perfusion conditions these results show remarkable agreement.

Placental performance. The maintenance and in some cases increment of physiologic maternal-to-fetal amino acid gradients (Fig. 5) show that this aspect of placental function, which requires intact membranes and adequate cellular energy, remains relatively unimpaired during perfusion.

In conclusion, our measurements that can be compared with other studies of the perfused human placental cotyledon (that is, rates of oxygen and glucose utilization, lactate synthesis, inulin clearance, and, insofar as other workers' results are available, adenine nucleotide concentrations) agree well, or favorably, bearing in mind different perfusion conditions. We also find, however, that energy levels of the perfused co-

tyledon do not recover from the rapid fall that occurs before perfusion restores an oxygen supply, although they do become relatively stable. In addition, the adenine nucleotide and lactate/pyruvate balances indicate the possibility of some metabolic differences from the in vivo state, which are likely to have some effect on placental function. These factors must be borne in mind when one is interpreting results from the preparation. Despite this, our results also suggest that the perfused cotyledon does, at least in certain respects, behave comparably with the in vivo state.

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Lymph node metastasis in cancer of the cervix: A preliminary report

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Accurate assessment of lymph node metastasis in cervical cancer is imperative to treatment plan. A histologic sampling method is suggested in which surgically excised lymph nodes are dissected at multiple levels before paraffin embedding. This approach proves to be a more sensitive procedure than the current bisection method in detecting metastasis. (*AM J OBSTET GYNECOL* 1986;155:388-9.)

Key words: Lymph node metastasis, cervical cancer, pathology technique

In cancer of the cervix, tumor metastasis to the lymph nodes adversely affects patient survival. The 5-year survival rate for Stage IB lesions is 90% if the lymph nodes are negative, whereas it ranges from 40% to 80% if they are positive.¹ The number of positive nodes, the unilateral or bilateral location of the positive nodes and the involvement of pelvic, para-aortic, or supraclavicular nodes all are related to the subsequent prognosis. Accurate assessment of the lymph node status is imperative in determination of the extent of disease and the type and extent of treatment.

The diagnosis of lymph node metastasis is based on

pathologic examination of the surgically excised lymph nodes. The present histologic method of sampling the nodes usually provides only a bisection of each node for microscopic examination (Fig. 1). Ahrens and Tschoke² reassessed lymph nodes by serial sectioning of the node-bearing paraffin blocks and found more than 100% diagnostic error (false negative) compared with the results of the original pathologic examination. Wilkinson and Hause³ demonstrated that further sampling of the nodes at different levels is associated with a significant increase in the incidence of positive nodes.

Given the facts that presence of positive nodes is determinative to treatment plan and patient survival and that further node sampling can increase the incidence of positive nodes, there is a great need to enhance the diagnostic accuracy of the current lymph node examination. A technique that is aimed at such an improvement must be routinely applicable in terms of labor intensiveness and cost effectiveness.

Past studies of lymph node investigation involved se-

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rial sections of the nodal blocks. This experimental approach demonstrated the presence of undetected metastasis in a precise, quantitative manner but poses two problems in routine practice. First, the number of sections to be screened per patient is prohibitively high. Second, for examination of various levels throughout a nodal block, the tissue ribbons sectioned between the levels would have to be mounted in order to keep a record of the tissue. This, per node, can number more than a hundred unnecessary slides.

Method and results

One potential approach to improve the sampling efficiency would be to dissect the node at various levels before paraffin embedding.³ This enables different levels of the nodes to be cut, mounted, and examined on one slide (Fig. 1). To evaluate the effectiveness of this approach, we carried out a pilot retrospective study on paraffin blocks of surgically excised lymph nodes from 35 patients with Stage IB lesions. All surgical excisions were carried out by senior gynecologic oncologists and past fellows to minimize variation in extent and quality of the nodal excision. Twenty of these patients had subsequent recurrence of disease and the remaining 15 were free of disease 5 years after the initial diagnosis. All of the original lymph node specimens were examined but different surgical pathologists carried out the examination. Thus all of the original slides stained with hematoxylin and eosin were reviewed by a single surgical pathologist to confirm the initial examination. The blocks were then dewaxed, and the nodes were redissected at multilevels, reembedded, sectioned, mounted, and reexamined microscopically (Fig. 1). The results indicated that three of the 20 patients with recurrence were each found to have one positive node, whereas none of the 15 disease-free patients was found to have a positive node.

This pilot study showed that three of 35 patients (9%) reported to have negative nodes actually had positive nodes, and three of 20 patients (15%) with subsequent

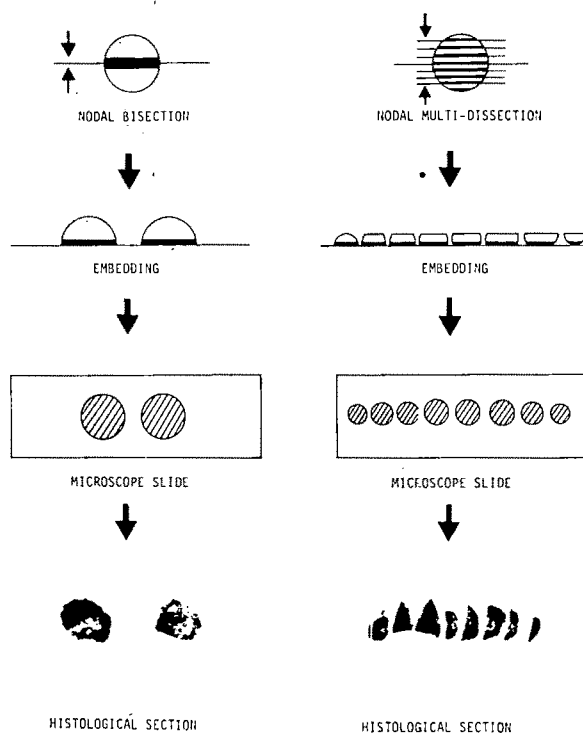


Fig. 1. Conventional lymph node bisection versus multilevel dissection.

disease recurrence had positive nodes. The false negative rate in either case is significantly high enough to warrant a larger series of study to confirm clinical utility before routine application.

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Elevated serum transaminase levels during ritodrine administration

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Two cases are presented of the occurrence of elevated serum transaminase levels during ritodrine administration for the treatment of premature labor. The clinical course of these cases suggests that elevated transaminase levels per se do not require the discontinuation of β -sympathomimetic tocolytic treatment. (AM J OBSTET GYNECOL 1986;155:390-2.)

Key words: Ritodrine hydrochloride, β -sympathomimetics, tocolysis, liver enzymes, transaminases

Recently, a case was reported relating the prolonged use of β -sympathomimetics to liver function impairment.¹ During treatment with terbutaline, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels increased progressively. For this reason the authors discontinued the tocolytic treatment despite expected fetal lung immaturity. Under similar conditions we decided to continue β -sympathomimetic tocolytic treatment.

Case reports

Case 1. A 35-year-old healthy, normotensive, white woman, gravida 3, para 2, was treated with oral ritodrine hydrochloride, 60 mg/day, from 26 weeks of pregnancy onward, because of threatened premature labor in the presence of chronic polyhydramnios. Fetal abnormalities, infections, diabetes, and blood group incompatibilities could not be demonstrated. At 32½ weeks' gestation she was admitted because of hydramnios and increasing uterine contractility in the presence of effacement and 3 cm dilatation of the cervix. Routine laboratory tests were normal, with SGOT and SGPT levels of 31 and 29 IU/L, respectively. After removal of 5 L of amniotic fluid by transabdominal amniocentesis during 6 hours the dosage of oral ritodrine was increased to 80 mg/day, and contractions ceased. The lecithin/sphingomyelin ratio of the amniotic fluid was 1.0. During the course of the following 2 weeks SGOT and SGPT values increased to reach a plateau at about

400 and 650 IU/L, respectively (Fig. 1, A). The patient complained of generalized itching not present earlier in gestation or in previous pregnancies, and serum bile acid levels were increased about twentyfold to 180 μ mol/L. Other indicators of liver function were virtually normal: bilirubin, 8 μ mol/L; creatinine phosphokinase, 48 IU/L; γ -glutamyl transpeptidase, 26 IU/L; lactic dehydrogenase, 414 IU/L; on ultrasound examination the liver showed a normal aspect. Pre-eclampsia and viral infections could not be demonstrated. At 35 weeks' gestation ritodrine treatment was discontinued because fetal lung maturity was expected to be adequate. During the following 2 days before delivery SGOT and SGPT values decreased to 170 and 406 IU/L, respectively. Vaginal breech delivery at 35½ weeks was uneventful and resulted in the birth of a healthy 3445 gm male infant with Apgar scores of 9 and 10 after 1 and 5 minutes, respectively. After delivery serum transaminase levels continued to fall. Microscopy of the placenta revealed less than 5% infarction and no signs of infection.

Case 2. A 32-year-old healthy, normotensive, white, primigravid woman with a twin pregnancy was admitted at 28 weeks' gestation because of threatened preterm labor. She used malaria prophylaxis, 100 mg of nivaquine daily, because of a recent stay in Africa. Ultrasound examination demonstrated an intact and well-developed twin pregnancy. Routine laboratory tests were normal; the SGOT and SGPT levels were 7 and 9 IU/L, respectively. Iron-deficiency anemia with a hemoglobin concentration of 5.5 mmol/L was successfully treated by transfusion of 2 units of blood followed by oral iron supplementation. Premature labor was treated with ritodrine hydrochloride intravenously, in a dose of 0.25 mg/min. Three weeks after initiation of this treatment SGOT and SGPT levels increased progressively to 340 and 750 IU/L, respectively (Fig. 1, B). The patient complained of severe and widespread itch-

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ing. Unfortunately, serum bile acid levels were not measured. The serum bilirubin level was increased to a maximum of 36 $\mu\text{mol/L}$ (normal < 12 $\mu\text{mol/L}$), and lactic dehydrogenase to 472 IU/L (normal < 350 IU/L); the other liver enzymes were within the normal range. On ultrasound examination the liver appeared to be normal. Preeclampsia, malaria, and viral infections could not be demonstrated. At 32½ weeks' gestation the lecithin/sphingomyelin ratio was 1.3, and the administration of ritodrine was augmented to 0.45 mg/min because of increased uterine contractility. Nonetheless, serum transaminase values did not rise any further during the next week and then fell gradually to reach normal levels at 36 weeks. At 36½ weeks' gestation she was delivered vaginally of two healthy male infants weighing 2640 and 2700 gm with good Apgar scores. On microscopy the placenta showed no abnormalities.

Comment

Two publications in the literature suggest a relationship between the use of β -sympathomimetics and liver function impairment.^{1,2} One report describes the occurrence of what is called "transient hepatitis" after a total oral dose of 160 mg of ritodrine hydrochloride administered in 36 hours.² Liver functions in this case were first reported as being abnormal after delivery, and coincidence rather than causality may have linked ritodrine to the liver disorder.

The other report describes markedly elevated serum transaminase levels in a patient during prolonged treatment for premature labor with a high dose of terbutaline.¹ Although the fetal lungs were expected to be immature, the drug was discontinued because of the progressive increase in SGOT and SGPT levels, with maximal values of about 350 and 600 IU/L, respectively, before delivery. Other biochemical liver functions were reported to be normal and viral infections could not be demonstrated. After withholding of the drug and subsequent delivery by cesarean section, SGOT and SGPT levels increased to 725 and 1010 IU/L, respectively. During the following days the transaminase levels returned to normal. In our first case, when ritodrine was withheld at 35 weeks of pregnancy, serum transaminase levels decreased markedly before delivery. This observation suggests even more strongly that a causal relationship between the use of β -sympathomimetics and elevated serum transaminase levels does indeed exist.

Because a low lecithin/sphingomyelin ratio suggested fetal lung immaturity we continued ritodrine therapy in both our patients despite initially progressive increases in SGOT and SGPT levels. Subsequently, in both patients serum transaminase levels reached a plateau. These elevated levels recovered quickly when

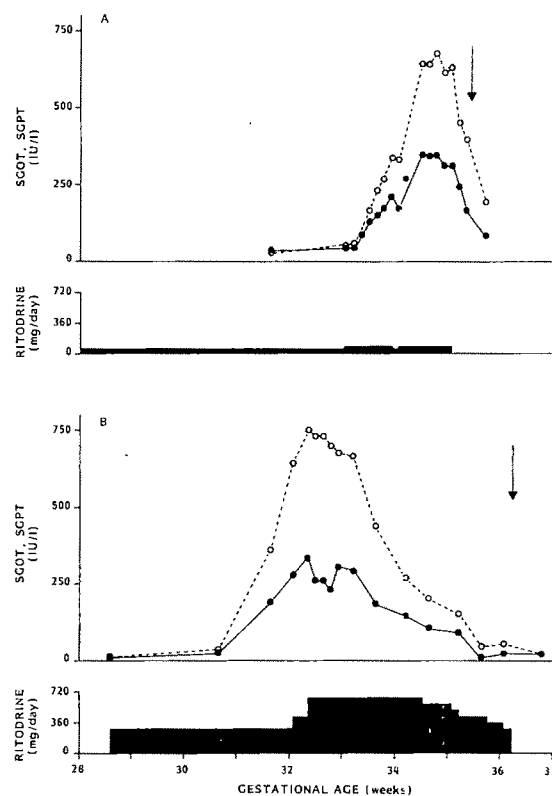


Fig. 1. Two cases of elevated serum transaminase levels during ritodrine hydrochloride administration. A: Case 1; B: Case 2. ●—●, SGOT; ○—○, SGPT; ▼, delivery.

treatment was discontinued in Case 1, while in the second case spontaneous recovery occurred during continued β -sympathomimetic treatment.

While β -sympathomimetics are widely administered, our report is the third that associates liver function impairment with their use. This suggests that such side effects occur infrequently and that other factors may also be of importance. The association of itching with elevated serum transaminase and bile acid levels, in the absence of major increases in other liver enzyme concentrations, bears some resemblance to cholestasis of pregnancy, the mechanism of which is equally unclear. One might speculate that the excessively enlarged uterus in both our cases has contributed to the liver impairment by a reduction in liver blood flow. In addition, our first case demonstrates that liver impairment can occur after the administration of a fairly low oral dose of ritodrine. Apart from individual sensitivity this could be due to the relatively high concentration of the drug in the liver after oral administration.

From our two cases we conclude that elevated serum transaminase levels may occur as a result of ritodrine administration. It does not seem necessary, however, to withhold the drug as long as tocolytic treatment

is indicated and other signs of liver dysfunction are absent.

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The value of serial human chorionic gonadotropin testing as a diagnostic tool in ectopic pregnancy

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This study examines the sensitivity of serial human chorionic gonadotropin determinations in the detection of ectopic pregnancy for patients who present with human chorionic gonadotropin titers <6500 mIU/ml. A log human chorionic gonadotropin time curve was used to calculate the slope of change. A slope below 0.11 (85% confidence limit for intrauterine gestations) is used as the cutoff value. Of 50 patients with serial titers at sampling intervals of 2 to 5 days, 26 (49%) had falling values and 22 had subnormal slopes. Only five had initially normal slopes and two of these patients eventually had falling titers. The sensitivity of an abnormal slope (below 0.11) in the detection of ectopic pregnancy was 90%. A 12.5% false positive rate was found in a group of 24 intrauterine pregnancies. (*AM J OBSTET GYNECOL* 1986;155:392-4.)

Key words: hCG, ectopic pregnancy, serial titers

The combined use of ultrasound and serum human chorionic gonadotropin (hCG) determinations has been suggested as a means of enhancing the specificity of intrauterine sonographic findings in the diagnosis of ectopic pregnancy.¹ The absence of a gestational sac when the serum hCG concentration is above 6500 mIU/ml is associated with ectopic gestations in 87% of cases.¹ The practical value of this criterion is limited to the 40% of patients with suspected ectopic gestations who present with an hCG titer above 6500 mIU/ml.¹

One of the management options in the evaluation of clinically stable patients presenting with hCG titers below 6500 mIU/ml is to perform serial hCG determinations. In a preliminary report, based mainly on retrospective data, we suggested that the log hCG time

curve or percent increase in hCG during a given sampling interval could be used as a screening technique for selecting patients for surgical diagnosis.² We now report the results of a 3-year prospective study with the use of this approach.

Material and methods

Between July 1, 1980, and June 30, 1983, there were a total of 244 surgically verified tubal ectopic pregnancies at Yale-New Haven Hospital. Of these patients, 74 had more than one hCG determination and an initial hCG concentration below 6500 mIU/ml. The logarithm of the serum hCG was plotted on the ordinate and sampling interval on the abscissa. Linear regression was used to calculate the slope of hCG increase. A slope of ± 0.11 was used to classify patients as having a normal or abnormal slope.²

Twenty-four patients with intrauterine pregnancies, who were originally thought to have ectopic gestations and were evaluated during a 10-month period, form the control group. This group was used to calculate the frequency of abnormal slope of hCG increase in cases of intrauterine pregnancies. All these patients had infants delivered at term.

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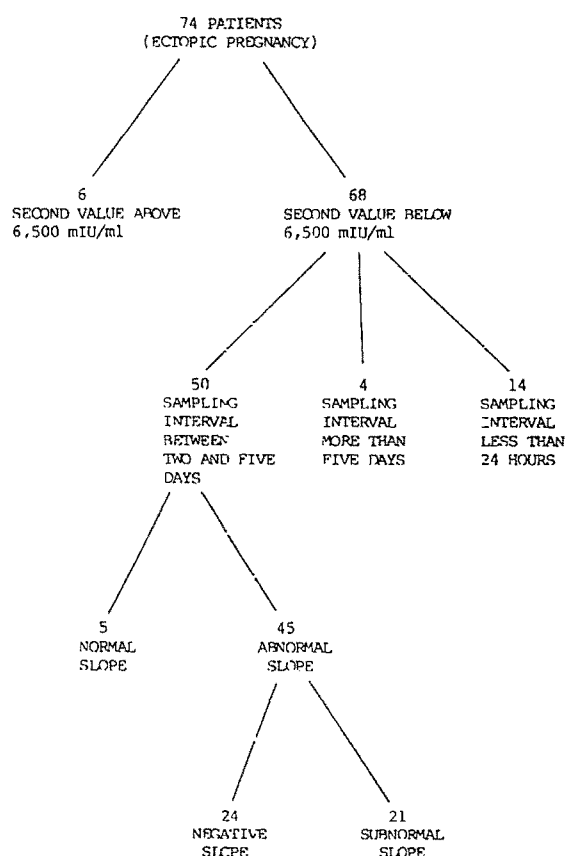


Fig. 1. Distribution of 74 patients with ectopic pregnancy and serial hCG determinations. Patients have been classified according to sampling interval and normality or abnormality of the slope of hCG increase with time.

Results

Of the 74 patients with ectopic pregnancies and serial hCG determinations, six had second values that were above 6500 mIU/ml; two of these had normal slopes and four subnormal. Sixty-eight patients had serial hCG determinations that were all below 6500 mIU/ml. In this group, 14 patients had a sampling interval that was only 24 hours, four patients had a sampling interval of more than 5 days, and 50 patients had a sampling interval that was between 2 and 5 days. Of the patients with a sampling interval of only 24 hours, two had normal slopes, three were subnormal, and nine had falling values and therefore negative slopes. Among the four cases with sampling intervals greater than 5 days, there were two with subnormal slopes and one with decreasing titers (Fig. 1).

There were 50 patients who had two or more samples taken at intervals between 2 and 5 days. There were five cases (10%) with a normal slope, but in two of these hCG values fell after initially normal increases. Of the remaining 45 cases, 24 had falling levels and 21 had subnormal rates of hCG increase. Fig. 2 depicts the distribution of the slopes in patients with ectopic ges-

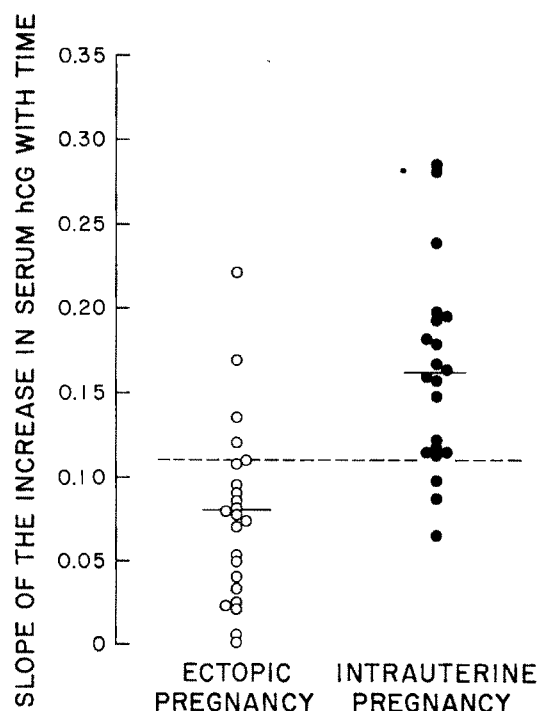


Fig. 2. Slopes of hCG with time in patients with intrauterine and ectopic pregnancy. Dotted line is placed at 0.11 while horizontal solid lines refer to the median slopes in patients with ectopic and intrauterine pregnancies.

tations with positive slopes and in those with intrauterine pregnancies. The mean slope for patients with ectopic pregnancies was 0.07 (SD = 0.05) and the mean slope for intrauterine pregnancies was 0.16 (SD = 0.05). These slopes were significantly different when compared with the use of a nonparametric test (Wilcoxon's test) ($p < 0.001$).

Among the 24 patients with suspected ectopic pregnancy that had normal intrauterine gestations, three had subnormal rates of increase (12.5%).

Comment

Previous work has suggested that interpretation of the sonographic findings in patients with suspected ectopic gestation is enhanced when used in conjunction with serum hCG levels. The absence of a gestational sac with levels above 6500 mIU/ml is associated with an ectopic pregnancy in 87% of the cases.¹ The limitation of this approach is that only 40% of patients with a suspected ectopic pregnancy will have an hCG titer above 6500 mIU/ml.¹

The options available to the physician for the management of the clinically stable patient with an hCG titer below 6500 mIU/ml include immediate surgical diagnosis with the laparoscope or follow-up of the patient with serial hCG determinations. The shortcoming of performing diagnostic laparoscopies on all patients

suspected to have ectopic pregnancies with titers below 6500 mIU/ml and no abnormal adnexal findings on ultrasound is that a high frequency of unnecessary operative procedures (up to 39%) will occur.³ Another alternative is to evaluate patients with serial hCG determinations. This communication examines the sensitivity and false positive rate of this approach.

The data set was subdivided according to the sampling interval; those patients with samples taken less than 2 days or more than 5 days apart were analyzed separately. This was because after only 1 day the difference between the mean percent hCG increase in intrauterine and ectopic pregnancies (20%) is less than twice the interassay variability. Long intervals (more than 5 days) raise questions about the pattern of hCG levels between the sampling points with potential effects on the accuracy of the calculated slopes.

Our data identify two main patterns of serial hCG determinations in patients with ectopic pregnancies: those with rising titers and therefore with a positive slope and those with falling hCG concentrations. Twenty-four patients (49%) with initial sampling intervals of 2 to 5 days had dropping hCG titers.

Our data indicate that an abnormal slope (below 0.11 or a negative slope) had a sensitivity of 90% (45 of 50; 21 with abnormal positive slopes and 24 with negative slopes) in the detection of ectopic gestation. When only positive slopes were considered, the sensitivity dropped

to 80.7% (21 of 26). The false positive rate with this criterion was 12.5% in the group of intrauterine pregnancies.

These data are of practical value in the clinical management of patients with suspected ectopic pregnancy with initial hCG values below 6500 mIU/ml and negative adnexal sonographic findings. These patients can be followed up with serial hCG titers. We have previously determined the lower limits of the percent increase of hCG per sampling interval. Under normal circumstances, hCG titers are expected to increase a minimum of 66% in 48 hours, 114% in 3 days, and 175% in 4 days. (Each of these rates of increase corresponds to a slope of 0.11). Using this approach, we could detect 90% of ectopic pregnancies. Using laparoscopy as the next diagnostic step, our data would suggest a 12.5% rate of unnecessary laparoscopy in patients so evaluated. This compares favorably with previously published management algorithms.

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A unique case of abdominal pregnancy: What are the minimal requirements for placental contact with the maternal vascular bed?

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In this report we describe a case of abdominal pregnancy in which the placenta engaged part of the wall of an intra-abdominal membranous sac with only minimal vascular connection with the peritoneal surface. A male infant weighing 3150 gm was delivered at 38 weeks' gestation. We believe this case represents the first report in the literature of an abdominal pregnancy without placental attachment to the maternal viscera. (*AM J OBSTET GYNECOL* 1986;155:394-6.)

Key words: Abdominal pregnancy, placenta, blood vessels

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The occurrence of ectopic pregnancy illustrates that the uterus itself is not compulsory in human reproduction. More than 95% of these pregnancies are found in the oviduct and are normally terminated by surgical intervention during the first 3 months of pregnancy. A

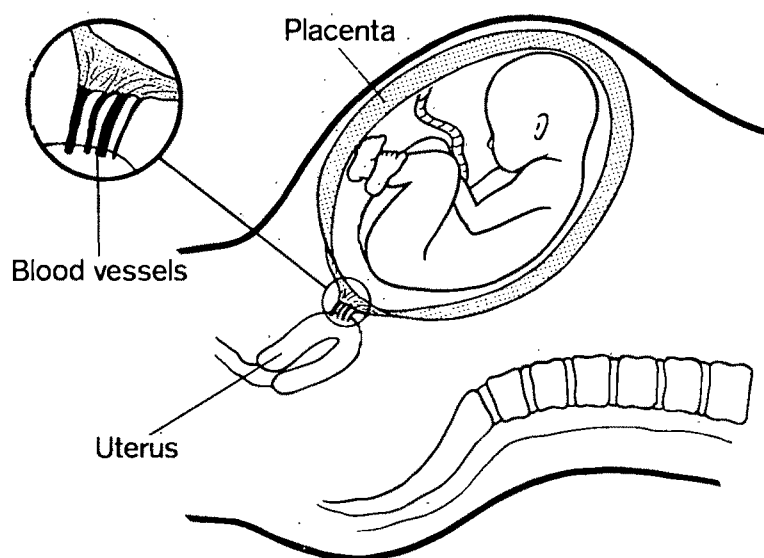


Fig. 1. A schematic illustration of the minimal vascular connection between the conceptus and the uterus at the time of delivery (magnification $\times$ upper left).

small proportion (2% to 4%), however, are located outside the fallopian tubes, and in turn approximately 10% of these are abdominal pregnancies, which may be associated with the delivery of a living child. In cases of abdominal pregnancy the placenta may be located in the pelvic area and usually has the character of a large irregular mass engaging the ovary, the broad ligaments, and the cul de sac. Alternative localizations are the omentum, the sigmoid bowel, and the small bowel, but the upper abdomen (the liver and the ventricle) may also be covered by placental tissue. Recently we have observed a case of abdominal pregnancy in which the placenta engaged part of the wall of an intra-abdominal membranous sac with only minimal vascular connection with the peritoneal surface. It is believed that this unique case may provide additional information concerning the biophysical demands for the establishment and development of human pregnancy.

Case report

A 30-year-old woman, gravida 5, para 2, with two spontaneous abortions and two living children, both delivered by cesarean section because of breech presentation, was initially hospitalized at 15 weeks' estimated gestational age complaining of pain to the right in the lower abdomen of almost 24 hours' duration. She also complained of nausea, vomiting, and loss of weight. At vaginal examination there was no sign of bleeding and the cervix was unripe. Ultrasound examination showed a living fetus and a myoma in the cervix 5 by 6 cm in diameter. The pain abated within 1 day and the patient was discharged with a diagnosis of normal intrauterine pregnancy. The feeling of general malaise, nausea, and vomiting continued during almost the whole pregnancy. At 29 weeks' gestational age ultrasound showed a total placenta previa and a

fetus in transverse lie. No myoma was seen on this occasion. For obvious reasons it was decided to deliver the fetus by cesarean section, which was performed in the thirty-eighth gestational week. At laparotomy an intra-abdominal pregnancy was found, the amniotic sac being composed of thickened chorioamniotic membranes with islands of placenta dispersed over almost the entire sac. A living male infant weighing 3150 gm without any deformities was delivered. Apgar scores were 8, 6, and 9 after 1, 5, and 10 minutes, respectively. The only connection between the mother and the conceptus was a stalk 2 cm wide implanted in the fundal area of the uterus about 1.5 cm medial to the right uterotubal junction. The stalk was approximately 3 cm long and contained four or five tortuous arteries and straight veins with an outer diameter of 4 to 7 mm. (Fig. 1). In a very simple manner the stalk was ligated and cut and the insertion point covered with peritoneum. The postoperative course was uneventful and the patient was discharged from the hospital 7 days later.

Histologic examination revealed a flat, outspread and partly membranous placenta with large intervillous thromboses and small infarctions. The umbilical cord showed a number of "false knots" but the vessels were normal without signs of vasculitis.

Comment

To our knowledge, this case represents the first report in the literature of an abdominal pregnancy without placental attachment to the maternal viscera. As mentioned initially the placenta is usually spread over a large peritoneal surface (for review see reference 1). In classical cases of intra-abdominal pregnancy, the removal of the placenta is associated with great technical difficulties and often severe hemorrhagic complications. In the actual case, however, the fetus and its surrounding placenta were in only minimal contact

with the uterus and the maternal circulation. The subsequent removal of the placenta after delivery of the fetus was thus remarkably simple.

The pathogenesis behind this case is of course uncertain and the possibility of a penetrating interstitial pregnancy or a primary peritoneal implantation must be considered. The criteria for primary peritoneal pregnancy proposed by Studdiford² are: (1) Both tubes and ovaries are normal with no evidence of recent or remote injury, (2) there is absence of any evidence of a uteroperitoneal fistula, (3) the pregnancy is related exclusively to the peritoneal surface.

The fact that this patient had undergone two cesarean sections earlier might give a suspicion for the presence of a uterine fistula. However, at both of these interventions the incision was made transversely in the lower uterine segment and there was no evidence of any complication. Moreover, at the present operation the underlying tissue at the insertion point in the uter-

ine fundus was clearly myometrium, which was confirmed by histologic examination.

It is thus reasonable to assume that this case represents a primary peritoneal pregnancy unique with regard to both its anatomy and the delivery of a living child at term. It seems remarkable that the fetus could survive and develop normally when the contact between mother and conceptus consisted of only four or five comparatively tiny vessels. Unfortunately it was impossible to assess the capacity of blood flow through the vessels.

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Advanced abdominal pregnancy associated with fetal pulmonary hypoplasia: Report of a case

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Delaying delivery of a premature abdominal pregnancy may allow for fetal pulmonary maturation. Prolonged extreme oligohydramnios, however, resulting in significant pulmonary hypoplasia would frustrate any effort to wait for pulmonary maturation. Little mention of this complication is found in the literature. Therefore, two cases of abdominal pregnancy are presented, one with fetal pulmonary hypoplasia and one without. Assessing the volume of amniotic fluid is suggested when a decision is being made to deliver this type of pregnancy. (*AM J OBSTET GYNECOL* 1986;155:396-7.)

Key words: Fetal pulmonary hypoplasia, abdominal pregnancy

Abdominal pregnancy accounts for about 1% of all cases of ectopic pregnancy. Because of the high rate of fetal mortality and severe maternal morbidity, delivery by laparotomy is usually performed whenever the diagnosis is made. However, when the fetus is alive and of 24 to 30 weeks' gestation and the mother is clinically stable, delaying delivery may allow the fetus to mature.¹

These babies can suffer pressure deformities of the skull and limbs because of the severe oligohydramnios that often complicates this condition. Only a single brief communication has reported fetal pulmonary hypoplasia associated with an abdominal pregnancy,² yet such a situation is ominous and would preclude waiting for fetal maturation. Two cases of advanced abdominal pregnancy are presented, one with fetal pulmonary hypoplasia and one without.

Case presentations

Case 1. R. M., a 26-year-old woman, gravida 1, para 0, had a diagnosis of abdominal pregnancy made when

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a laparotomy was performed for failure to progress in labor at term. Despite three sonographic studies at 6, 20, and 34 weeks' gestation the extrauterine nature of the implantation went unrecognized. The baby appeared to have normal growth and development and was surrounded by a normal amount of amniotic fluid. The patient developed an acute abdomen at 37 weeks' gestation and underwent a laparotomy. A completely normal term infant was found surrounded by amniotic membranes and a normal amount of amniotic fluid. The placenta was attached to the anterior surface of the uterus and was removed by means of an uneventful hysterectomy. The baby did well.

Case 2. D. B., a 34-year-old woman, gravida 2, para 1, had an abdominal pregnancy diagnosed by a sonogram at 29 weeks' gestation. The baby was in the upper abdomen of the mother and was surrounded by no amniotic fluid, yet within the limits of the sonographic evaluation development of the head, thorax, and limbs appeared to be normal. The fetal heart rate pattern revealed frequent variable decelerations but was otherwise normal for a 29-week pregnancy. Nonstress testing was nonreactive, and an oxytocin challenge test was not possible. A biophysical profile produced a score of 2 of a possible 10, although the relevance of the criteria used in this clinical setting was questioned. The mother was given a course of steroids and the baby continuously monitored until delivery was accomplished by laparotomy 48 hours later. The infant was located in the abdomen of the mother and had no surrounding amnion or amniotic fluid. The gestational age was 29 weeks by the Dubowitz criteria, and the baby had pressure deformities of the face and club feet. The infant's neonatal course was complicated by extreme pulmonary insufficiency. Despite mechanical ventilation with high pressures and oxygen tension, adequate oxygenation was never achieved. The thoracic cavity was abnormally small and an adequate inspiratory volume not obtained even with ventilatory support. A diagnosis of pulmo-

nary hypoplasia was made by the neonatologists. The baby died of this condition 5 days after birth. The placenta was found attached to the left posterior broad ligament and lateral pelvic sidewall and was completely removed without hysterectomy.

Comment

Timing delivery of the abdominal pregnancy with a living infant at 24 to 30 weeks' gestation is complex. If the mother and infant appear stable, efforts to postpone delivery and allow time for fetal pulmonary maturation may result in a satisfactory neonatal outcome that would otherwise be complicated by severe hyaline membrane disease and other problems suffered by the extremely premature baby. In the second case, however, because of pulmonary hypoplasia of the infant probably resulting from the total lack of amniotic fluid, delaying delivery would not have accomplished further pulmonary maturation.

We suggest that in similar situations the obstetrician pay close attention not only to the clinical status of the mother and infant but also to the volume of amniotic fluid surrounding the baby. If a reasonable amount of fluid can be visualized, then delaying delivery may improve the neonatal outcome. However, if no amniotic fluid can be seen despite careful sonographic evaluation, heroic efforts to delay delivery may only be frustrated by the lack of normal pulmonary development of the infant.

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Successful midtrimester thoracentesis with analysis of the lymphocyte population in the pleural effusion

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A case of successful midtrimester fetal thoracentesis is reported. Several chest taps were performed in a fetus between 20 and 23 weeks' gestation to relieve the large and progressing pleural effusion. Analysis of the lymphocyte subpopulations in the pleural fluid showed differences in cell mix when compared with that of our previous, equally successful reported case. (*Am J Obstet Gynecol* 1986;155:398-9.)

Key words: Fetal thoracentesis, pleural effusion, chylothorax

During the past several years, in utero manipulations of fetal anomalies have been possible under ultrasound guidance, although only in a few cases has there been probable benefit shown. In this report, we describe a patient who underwent five in utero fetal thoracenteses between 20 and 23 weeks' gestation, with complete resolution of the pleural effusion by 26 weeks. We reported a similar case recently, with an equally successful outcome.¹ Analysis of the lymphocyte subpopulation in the pleural fluid in these two cases revealed substantial differences; however, the presumptive diagnosis in both cases was chylothorax, and both fetuses responded similarly to the therapy. To our knowledge, these two patients represent the only reported cases of fetal thoracentesis in the second trimester.

Case report

L. K., a 37-year-old white woman, gravida 1, para 0, had an amniocentesis at 16 weeks with a yield of normal chromosomes. A left fetal pleural effusion was noted and was unchanged at 17, 18, and 19 weeks. At 20 weeks, the amount of fluid in the left side of the fetal chest increased dramatically, shifting the heart toward the right side of the chest, and a small right effusion was noted. At that time, a 25-gauge spinal needle was passed into the left side of the fetal chest and 12 ml of straw-colored fluid was withdrawn (Fig. 1). During the next week, the fluid reaccumulated with a large shift of the mediastinum, and a transient rim of ascites ap-



Fig. 1. Transverse view of the fetal chest through the back with the spine shown by the open arrow. The 25-gauge needle is seen entering the fetal left hemithorax posteriorly (the shaft of the needle is indicated by the small arrows and the tip by the large solid arrow).

peared. At 21, 21.8, 22.5, and 23 weeks 13 to 17 ml of fluid was withdrawn from the left side of the fetal chest without complication. The samples of pleural fluid obtained were analyzed for lymphocyte cell type. By 24.5 weeks, the pleural effusion could barely be seen and by 26 weeks it was gone. At term, the patient was delivered of a healthy baby girl who had a normal physical examination, showed no evidence of thoracic or chest abnormalities, and needed no further therapy.

Comment

Of the many fetal abnormalities diagnosable by ultrasound, only a few are currently to be considered for in utero manipulation, and it is often difficult to prove

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a benefit from this intervention. In the case reported here, the fetal condition was worsening visibly, with an enlarging left pleural effusion, causing a marked shift of the mediastinum and deviation of the heart. The appearance of a small right pleural effusion and a thin rim of ascites at 22 weeks suggested that, without decompression of the left hemithorax, there might be progression to more severe hydrops. This process reversed with our therapy.

In an unreported case from our own laboratory several years ago, we observed a fetus with similar sonographic findings at 20 weeks, and without in utero therapy, this fetus developed hydrops and ascites and died.

An important part of fetal lung development is known to take place in the second trimester and relieving intrathoracic pressure may be crucial during that time for proper lung growth. Fetuses with chest masses, such as diaphragmatic hernia, in utero are at high risk for hypoplastic lungs, and the mechanism of chest compression may be similar to that observed with oligohydramnios.

When the present case is compared with our previously reported case of thoracentesis between 19 and 22 weeks' gestation, striking similarities are evident.¹ The gestational age at which the pleural effusion was at its worst was almost identical and the age at which it no longer accumulated was also very close. The cell type in the pleural fluid was surprisingly different although the pleural fluid in both cases contained 90% mononuclear cells. The subpopulation of lymphocytes in our previously reported case was exclusively T lympho-

cytes. In our present case there was a mixed cell type with the lymphocyte distribution resembling that seen in umbilical cord blood (mixed T and B lymphocytes). Studies using extensive thoracic duct drainage to promote cardiac allograft survival in rats have shown that the presence of B lymphocytes is not an uncommon finding in the rat thoracic duct.² Therefore, the presence of B lymphocytes in pleural fluid from one fetus but not the other cannot be used to determine cause or to differentiate between thoracic duct injury and an exudative process.

The five procedures performed in this patient were done with a 25-gauge spinal needle with continuous sonographic guidance and only once were two passes needed to enter the fetal chest. No complications were seen from any of the procedures and the 25-gauge needle was felt to be atraumatic.

The case described here, together with our prior experience, suggests that this abnormality can easily be successfully treated with a number of drainage procedures and that not every chylous effusion or pleural effusion will have the same distribution of lymphocyte subtypes.

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Failure of ibuprofen in treatment of herpes genitalis

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Motrin (ibuprofen), a potent inhibitor of prostaglandin synthetase, was tested in women and men for treatment of recurrences of genital herpes. In a double-blind trial, no statistically significant differences were observed between treated and placebo groups in any of the measured parameters. (AM J OBSTET GYNECOL 1986;155:399-400.)

Key words: Herpes simplex, ibuprofen, recurrence

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Table I. Virus titer, lesion area, and presence of pain in experimental subjects

	Day 1	Day 2	Day 3	Day 5	Day 7
Titer (pfu/ml) (mean $\pm$ SEM)					
Motrin	$2.1 \pm 1.1 \times 10^3$	$0.5 \pm 0.3 \times 10^3$	$1.6 \pm 1.5 \times 10^2$	$1.1 \pm 0.9 \times 10^2$	0
Placebo	$3.5 \pm 2.4 \times 10^3$	$5.1 \pm 2.6 \times 10^3$	$0.3 \pm 0.2 \times 10^2$	$1.4 \pm 1.2 \times 10^2$	$4.9 \pm 5.0 \times 10^1$
Total lesion area (mm ²) (mean $\pm$ SEM)					
Motrin	7.9 ± 0.6	7.8 ± 1.1	6.9 ± 0.9	3.9 ± 1.0	2.3 ± 1.0
Placebo	8.2 ± 1.3	9.8 ± 2.3	6.3 ± 1.6	3.9 ± 1.4	3.9 ± 1.4
Pain present (% of subjects)					
Motrin	84.6	81.8	60.0	25.0	27.3
Placebo	85.7	75.0	57.1	46.2	30.8

pfu = Plaque-forming units.

Experimental evidence indicates that prostaglandins may play a role in the activation and cell-to-cell spread of recurrent herpes simplex virus infections. Therefore prostaglandin inhibitors may be effective in controlling such infections.^{1,2} The present study was undertaken to assess the efficacy of Motrin (ibuprofen), a nonsteroidal anti-inflammatory agent and prostaglandin synthetase inhibitor, on the duration and severity of herpes simplex virus recurrences.

Material and methods

Twenty-seven otherwise healthy male and female volunteers (aged 21 to 44, mean 32.1 ± 6.2 years) with documented recurrent genital herpes simplex virus infections were enrolled in the study. The protocol was previously approved by the Institutional Human Studies Committee. Patients were assigned to Motrin or placebo groups in a double-blind fashion. They received the study medication in advance and initiated treatment on the first sign of recurrence. Those in the Motrin group received Motrin, 600 mg three times a day for 5 days. The placebo group received the medication in the same fashion. Patients presented at the clinic within 24 hours of the appearance of lesions (day 1) for cultures and blood test and on days 2, 3, 5, and 7 for cultures only. Total lesion area, stage of lesion, and presence or absence of pain were noted. Herpes sim-

plex virus was cultured and titered on Vero cells. Statistical analyses were by Student's unpaired *t* test.

Results and comment

Although differences between the treated and placebo groups were present and occasionally quite pronounced, none of the differences were statistically significant. Viral shedding lasted 2.6 ± 0.4 and 3.6 ± 0.9 (mean $\pm$ SEM) days in the Motrin and placebo groups, respectively. Crusting of lesions required 4.9 ± 0.5 and 4.8 ± 0.7 days in the same groups. Table I shows the titer of viral isolates, total lesion area, and presence of pain during the trials. No statistically significant differences were observed. Therefore, in clinically recurrent genital herpes, Motrin cannot be considered the treatment of choice.

We thank Ms. Georgia Brown for her assistance with this project.

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Current Development

The placental bed biopsy: Review from three European centers

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This review derives from extensive experience with the placental bed biopsy technique in three centers over the last 30 years. A placental bed biopsy, usually taken at cesarean section, must include basal decidua and subjacent myometrium from the central zone of the placental site. Attention is drawn specifically to the sampling errors and to the pitfalls in morphologic interpretation of tissues, both maternal and fetal, that are continuously changing throughout the course of pregnancy. The features of the normal placental bed and of vascular lesions in pathologic pregnancies are briefly reviewed. Extension and elaboration of the technique and its more widespread use could contribute to the elucidation of many of the unresolved problems in human pregnancy. (AM J OBSTET GYNECOL 1986;155:401-12.)

Key words: Placental bed, placentation, preeclampsia, fetal growth retardation, trophoblast

Until relatively recently morphologic studies of human placentation in normal and abnormal pregnancy were based on the delivered placenta, on sporadic hysterectomy specimens during pregnancy, and on specimens derived at autopsy after maternal death. The maternal side of the placenta, particularly in pregnancy disorders, remains something of an enigma as postpartum involution of the uterus ablates any evidence of defective placentation that may be a determinant of abnormal pregnancy.

Histologic studies of human hemochorial placentation must include material deep in the placental site as it is there that two important phenomena occur, one being the controlled invasion of maternal tissues by migratory extravillous trophoblast and the other the conversion by these cells of maternal spiral arteries to uteroplacental arteries supplying blood to the intervillous space of the placenta.¹⁻³

In 1958, Dixon and Robertson,¹ in an investigation of the hypertensive disorders of pregnancy, introduced a new technique for obtaining tissue from the pregnant uterus at cesarean section, which they called "the placental bed biopsy." The word bed was chosen deliber-

ately, instead of site, as the authors wished to emphasize that the biopsy must include not only basal decidua but also underlying myometrium containing the origins of the uteroplacental (spiral) arteries. These vessels, particularly in hemochorial placentation, are likely to be the targets of lesions in such disorders as hypertension, intrauterine fetal growth retardation, and renal disease where there is evidence of impaired uteroplacental blood flow.^{3-11*} It is also probable that in many other disorders of pregnancy, such as spontaneous abortion, antepartum hemorrhage, and systemic diseases complicating pregnancy, such as diabetes mellitus, the uteroplacental circulation is directly or indirectly affected.¹³

The use of the placental bed biopsy technique as part of the investigation of placentation in normal and abnormal pregnancy has proved to be safe and is now well established with a sizable literature on the subject.^{2, 4, 12-35} It is our feeling, however, that the value of some reported studies is vitiated by failure on the part of investigators fully to appreciate the limitations of the technique, the sampling problems, and the inherent interpretative difficulties. This has resulted in conflicting reports leading to confusion among readers who are unfamiliar with the technique and those with little experience of morphologic studies. It is timely therefore to review all aspects of the technology to highlight these limitations and to encourage accuracy and precision in the interpretation of placental bed biopsies.

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Fig. 1. Well-oriented placental bed block with decidua at top and myometrium below. The myometrial and decidual segments of a uteroplacental (spiral) artery with physiologic changes are shown. (Hematoxylin and eosin. Original magnification $\times 50$.)

Taking the biopsy specimen

Two methods are available to obtain tissue, the first at cesarean section and the second vaginally immediately post partum. The second method, in which specially adapted ovum or other forceps is used, obviously will allow biopsies to be taken in large numbers of cases but it is less reliable in terms of sampling of the placental bed³⁰ even though the precise placental site may have been determined by ultrasonography during pregnancy. Furthermore, the risks of biopsy via the vagina, potential or real, make it a dubious ethical procedure to undertake when no therapeutic benefit accrues directly to the patient or her baby. Only when cesarean section is not indicated or is not relevant, as in cases of recurrent abortion, should biopsies be taken vaginally, when otherwise material for study of possible abnormal placentation would be denied to the investigator.

Biopsies at cesarean section are taken under direct visualization of the placental site. After delivery of the baby the true center of the placenta, not the insertion of the cord, should be digitally marked by the assistant and the placenta peeled away from its bed by the operator. It will then be seen that the placental site is somewhat depressed, focally disrupted, and friable

when compared with the smooth but undulating surrounding decidua parietalis or vera. Careful inspection of the placental site and its surroundings and increasing experience of the interested obstetrician will allow the distinction between the two to be made and also recognition of the sheared uteroplacental vessels, including dark red thrombi when present in those vessels. With curved scissors a disk of about 1.5 cm in diameter, to include basal decidua and underlying myometrium, is removed from the central zone of the placental bed and from other areas suggestive of possible abnormality such as vascular lesions. It is emphasized that there is no need to cut deeply into the myometrium; a few millimeters suffices as the myometrial segments of the spiral arteries are contained within that depth. Hemostasis, if thought necessary, can be secured by absorbable sutures. Difficulty may be experienced when the placenta is in an anterofundal site, but if the uterus is partially exteriorized and the upper lip of the lower segment incision is everted by an assistant, adequate access to an anterior placental site can be achieved.

To avoid the potential crushing artifacts produced by scissors, biopsies may also be taken with nontoothed forceps and scalpel to produce a wedge-shaped piece of placental bed measuring approximately 1.5 cm in length, 0.5 cm in depth, and 1 cm in breadth at the decidual surface.²² The resulting defect is closed with absorbable sutures.

In experienced hands the true placental bed can be sampled in more than 70% of cases.² Taking random blind biopsies with adapted cutting forceps generally results in inadequate material and tissue from outside the placental bed in a high percentage of cases.

Processing the biopsy specimen

The handling of biopsy specimens will depend on the objectives of the particular investigators, that is, whether they are interested only in diagnostic assessment or morphologic research studies at the light or electron microscopic levels, in histochemical or immunocytochemical studies, or in various combinations of these.

If the biopsy specimen is to be examined only by routine light microscopy it should be placed immediately with the myometrial aspect down on a piece of filter paper, in the manner oral jejunal biopsy specimens are handled, and allowed to adhere for a few seconds. The excess filter paper is then cut away and the portion carrying the biopsy specimen is inverted and placed in 4% buffered neutral formaldehyde-saline solution (so-called 10% formal-saline) or another appropriate fixative such as Bouin's solution. After 12 to 24 hours of primary fixation, the biopsy specimen can then be cut into strips of about 3 mm breadth, each strip being properly oriented in the ultimate wax block

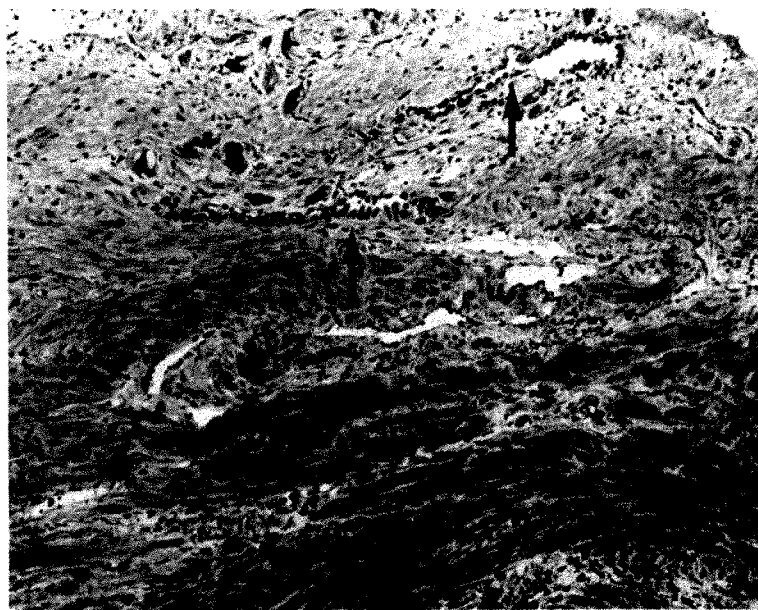


Fig. 2. Frozen section of placental bed biopsy specimen with characteristic giant cells. Note the endometrial glands (*arrows*) with flattened but still prominent epithelium in the decidua. (Hematoxylin and eosin. Original magnification $\times 125$.)

to yield perpendicular sections from the decidual surface to the myometrial base (Fig. 1). Proper orientation of the biopsy specimen is absolutely essential for the accurate assessment of topographic relationships as it must be appreciated that the junction of decidua with myometrium is ill-defined, there being no muscularis mucosae to separate the two.

If the biopsy specimen is also to be used in cryostat work then half the tissue on the filter paper should be snap frozen immediately in liquid nitrogen. Tissues to be frozen for lipid, histochemical, and immunocytochemical studies should not be subjected to solid carbon dioxide or freezing-spray treatment as these agents are prone to cause ice crystals and other artifacts.

Where ultrastructural study is the main objective the whole biopsy specimen should be placed immediately, in the operating room, into cold, cacodylate-buffered glutaraldehyde solution and quickly transported to the laboratory (see below).

Special histologic techniques. Paraffin sections may, of course, be stained with a variety of dyes to define and highlight specific tissues and substances such as elastica, fibrin, fibrinoid, collagen, connective tissue, smooth muscle, glycogen, and mucins and also for certain immunocytochemical techniques adaptable to paraffin sections. In all circumstances, however, it is essential that the biopsy specimen is first assessed by a routine stain such as hematoxylin and eosin, preferably supplemented by the periodic acid-Schiff technique.

When cryostat sections are to be used for histochemical, immunofluorescence, and immunocytochemical

work the same rule applies. The first adequate section should be stained with hematoxylin and eosin and here those unfamiliar with frozen sections of the placental bed may experience interpretative difficulties. It is a question of familiarization with a different set of artifacts. For instance, the basal endometrial glands are much more prominent in a frozen section (Fig. 2) than in a paraffin section; in the latter they may go unnoticed or, because of shrinkage of the already flattened epithelium, be misinterpreted as veins or lymphatics. Slow and inadequate freezing of tissue leads to ice crystal artifact on sectioning and the spongy decidua is particularly prone to this blemish.

When the uteroplacental vasculature is specifically being studied numerous sections may have to be cut before a satisfactory picture of a vessel is seen because of their tortuous nature, which may result in a uteroplacental artery being missed in a single or random section. Furthermore, a tangential cut may give misleading information such as apparent absence of trophoblast-induced vascular changes or a false impression of hyperplastic changes with luminal stenosis or even occlusion (Fig. 3).

Electron microscopy. Biopsy specimens partially fixed in cacodylate-buffered glutaraldehyde for ultrastructural studies are sliced into segments 2 to 3 mm wide perpendicular to the decidual surface on receipt in the laboratory. A dissecting microscope can be used to aid distinction of the more friable decidual layer from the meatier myometrial tissue and the resultant strips are immersed for a further 1½ hours in fresh



Fig. 3. Tortuous uteroplacental artery at the deciduomyometrial junction. The lower segment has been cut tangentially giving an artifactual impression of intimal hyperplasia with luminal stenosis. (Hematoxylin and eosin. Original magnification $\times 125$.)

fixative, the container being placed on ice and in a 4°C refrigerator. Following three washes at hourly intervals in cacodylate buffer the strips are examined under a dissecting microscope and blocks of tissue 2 to 3 mm³ are cut to contain, whenever possible, a blood vessel with a known orientation in the block (Fig. 4). The blocks are then postfixed in osmium tetroxide and processed in resin by standard methods. Semithin sections are cut and suitably stained to check light microscopic features and orientation of blood vessels before ultrathin sectioning.

Morphologic features of the normal placental bed

The morphologic features of the normal human placental bed are now reasonably well documented^{2, 3, 36-38} so that only the important and relevant features will be discussed here. The phenomenon of migratory extravillous trophoblast is undoubtedly the key to an understanding of the morphologic features that evolve throughout pregnancy, yet little is known about the mechanisms that promote and control this invasion of maternal tissue by fetal cells. In the first instance, cells proliferating from the cytotrophoblastic shell surrounding the conceptus in the earliest stage of gestation



Fig. 4. Block from wedge-shaped placental bed biopsy specimen taken for electron microscopy. Note the distended vascular lumens (arrows) particularly in the decidual component (top). ($\times 6$.)

and later from the tips of the anchoring villi move rapidly through the basal decidua to reach the inner layer of the myometrium before the end of the first trimester.³⁶ Thereafter, by symplasmic fusion, this extravillous trophoblast gradually converts to the syncytial placental bed giant cells that are characteristic of the second and third trimesters. The functions of this interstitial form of extravillous trophoblast remain to be determined.

A second, more easily observed property of migratory trophoblast is the opening up of the maternal spiral arteries in its path that gradually convert these small caliber arteries in the placental bed to large, tortuous sinusoidal vessels of low resistance, the uteroplacental arteries that open into the intervillous space. It was to this pregnancy-induced vascular adaptation that Bro-sens et al.² gave the term "physiologic changes."

The walls of these uteroplacental arteries gradually lose their normal musculoelastic structure ultimately to become flaccid tubes composed of fibrinoid material and fibrous tissue in which trophoblastic cells are embedded (Fig. 5). There is now evidence,³⁹ supported by analogies from rodent placentation,⁴⁰ that the transformation of the spiral arteries in the placental bed to uteroplacental arteries is achieved in two stages. The

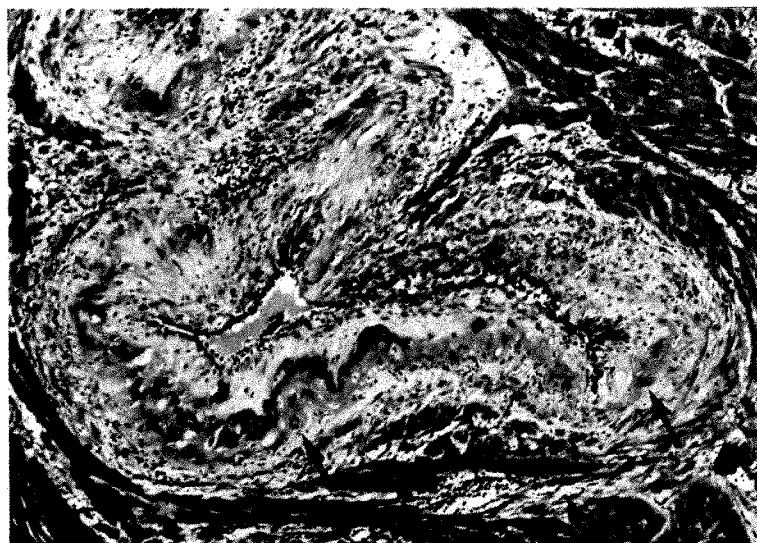


Fig. 5. Fully developed physiologic changes in the myometrial segment of a uteroplacental artery. There is a prominent convoluted layer of fibrinoid in which discrete trophoblastic cells are embedded (arrows). Note that the vessel is artifactually collapsed. (Hematoxylin and eosin. Original magnification $\times 125$.)

first phase occurs in the first trimester in the decidual segments and the second, mediated by a further wave of endovascular trophoblast migration during the second trimester, leads to the conversion of the myometrial segments of these arteries.

Because the openings of the uteroplacental arteries, ultimately some 100 to 150 in total¹¹ apparently are random in distribution, there is a formidable sampling problem. However, because these vessels are tortuous in their course from myometrium through the decidua, segments of vessels should be seen in biopsy specimens from normal pregnancies if the biopsy is about 1.5 cm² or more in its decidual surface area. Smaller arteries, <120 μ m in diameter without physiologic changes, are identifiable in decidua and myometrium but these are basal or "straight" arteries supplying myometrium and decidua only and not the fetal placenta. The basal arteries must not be classified as uteroplacental arteries without physiologic changes.

The determination as to whether a biopsy is from the central or peripheral zones of the placental bed is not easy and it must largely depend on the extent and density of placental bed giant cells in the basal decidua and myometrium. Absence of such cells from the myometrium almost certainly indicates marginal areas of the placental bed. During the establishment and development of placentation in the first two trimesters there is a "fading" of interstitial trophoblast migration and of trophoblast-induced physiologic changes in the spiral arteries centrifugally from the original nidation site.³⁷ This means that the marginal zone will show extravillous trophoblast only in the decidua and physiologic vascular changes only in the decidual segments

and not in the myometrial segments of the spiral arteries. Failure to appreciate the significance of these features can lead to misinterpretation, particularly in relation to pathologic pregnancies.

From these observations it follows that an absolutely essential first step in the histologic assessment of routine sections of a biopsy is to determine whether the tissue is from the true placental bed or from the decidua parietalis with its underlying myometrium. Even if the former, then an attempt must be made to assess whether the biopsy specimen is from the central zone of the placental bed or from the periphery. The following are the criteria for identification of the normal placental bed in the third trimester: (1) There is present, in the decidua and in the myometrium, extravillous trophoblast, that is, mononuclear cytotrophoblast, which is scanty in the third trimester, and syncytial placental bed giant cells, the predominant trophoblastic element in the third trimester (Fig. 6). (2) Retained anchoring villi on the fibrinoid decidual surface are a helpful feature, but care must be exercised not to be misled by non-placental bed biopsy specimens contaminated by fragments of placental tissue (Fig. 7). In the latter case, the absence of extravillous trophoblast in the decidua provides the clue. (3) When included in the biopsy, the characteristic physiologic changes in decidual and myometrial segments of the spiral (uteroplacental) arteries are the most striking feature distinguishing placental bed (Fig. 5) from non-placental bed (Fig. 8) biopsy specimens.

In cases of early pregnancy, when biopsies are taken only vaginally or very occasionally at hysterotomy, their characterization must depend on the presence or ab-

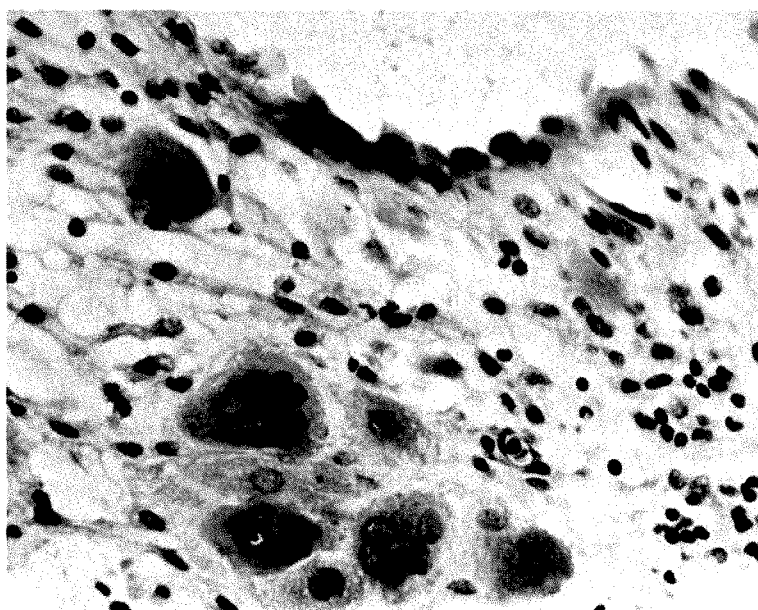


Fig. 6. Placental bed giant cells adjacent to an endometrial gland. Note also mild round cell infiltrate in the decidua. (Hematoxylin and eosin. Original magnification $\times 500$.)

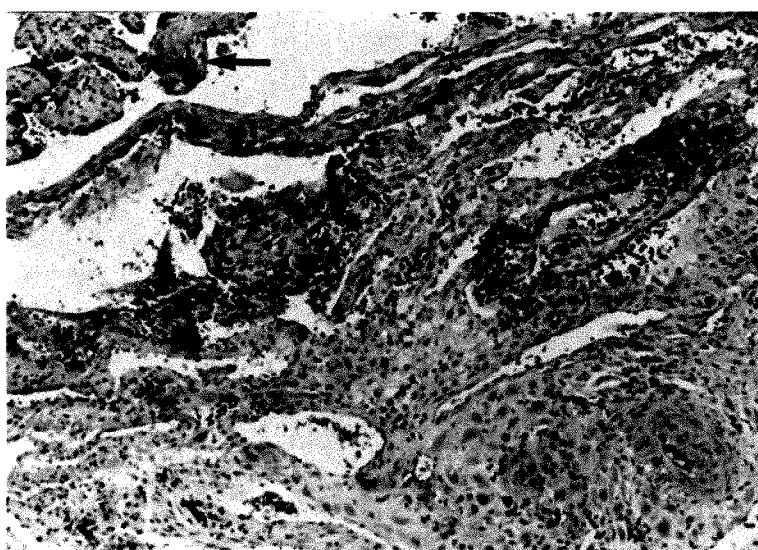


Fig. 7. Non-placental bed biopsy (decidua vera or parietalis) contaminated on its surface by a few placental villi (*arrow*). There are no trophoblastic elements in the decidua. (Hematoxylin and eosin. Original magnification $\times 125$.)

sence of extravillous trophoblast in the decidua but, even in these, the early phases of physiologic changes in decidual vessels usually can be detected (Fig. 9).

Pathologic features of the placental bed

Most morphologic studies of the placental bed to date have been concentrated on the uteroplacental vasculature in normal and abnormal pregnancies.^{2, 16, 18, 22, 24, 25, 30, 34} More recently attention has been directed also to the migratory pattern and function of

extravillous trophoblast,³⁶⁻³⁸ including immunologic aspects.⁴²⁻⁵² A brief survey of the current status, to include areas of controversy and ignorance, is therefore in order.

Inadequate physiologic changes. There is now good evidence that in the hypertensive disorders of pregnancy, particularly where there is associated intrauterine fetal growth retardation, the extent of physiologic changes in the uteroplacental arteries is restricted,¹⁸ implying that the second wave of endovascular tro-

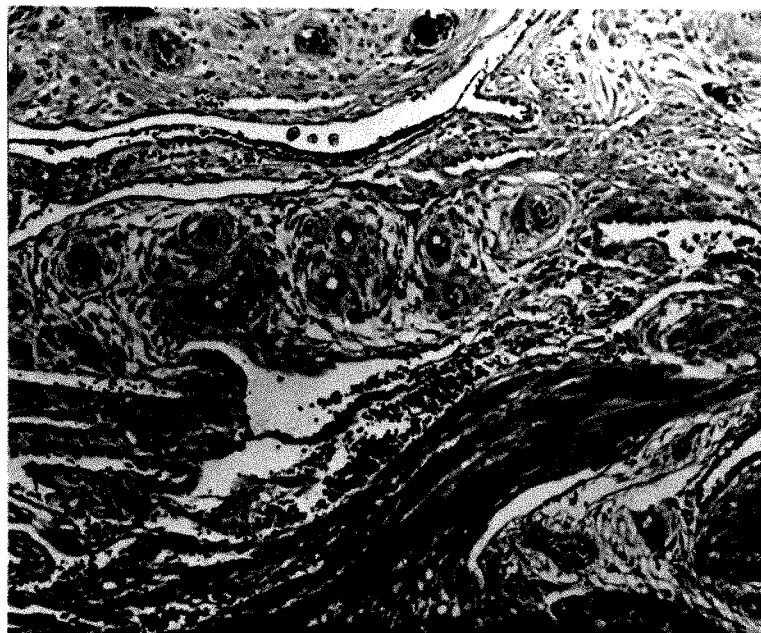


Fig. 8. Biopsy specimen from the nonplacental bed including decidua vera and underlying myometrium. There are no trophoblastic cells and the spiral arteries show no evidence of physiologic changes. (Hematoxylin and eosin. Original magnification $\times 125$.)

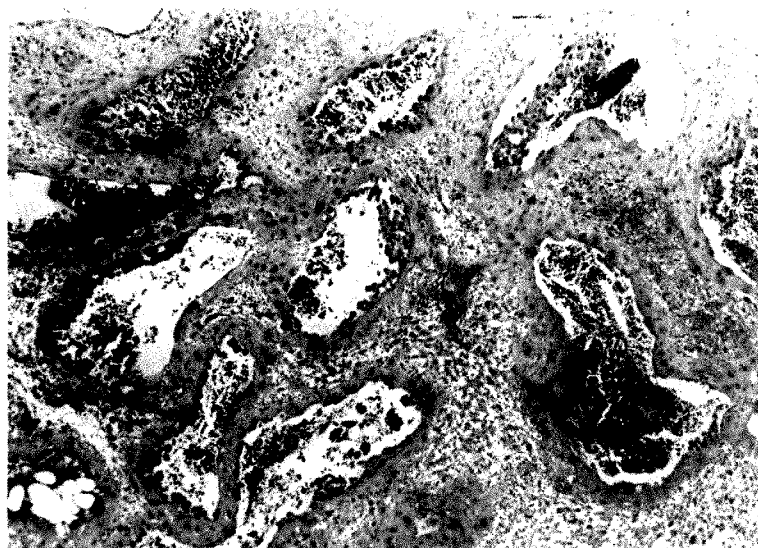


Fig. 9. Curettage specimen after spontaneous abortion at 10 weeks' gestation. The basal decidua contains numerous cuts through developing uteroplacental arteries, some of which contain luminal endovascular trophoblast. (Hematoxylin and eosin. Original magnification $\times 50$.)

phoblast migration into the myometrial segments of the spiral arteries is inhibited.^{12, 24, 25, 30, 34, 39} Similarly, in intrauterine growth retardation unassociated with maternal hypertension, the maternal vascular component of hemochorial placentation may be less than adequate.^{12, 18, 24, 25, 26, 31, 33} Difficulties arise in substantiating these postulates largely because of the lack of uniformity of definitions of the conditions under study.^{34, 35} Certainly, in the more severe cases of the preeclampsia-

eclampsia syndrome, where significant proteinuria is a feature, it is generally accepted^{12, 18, 30, 31, 34, 39} that the physiologic changes are restricted to the decidual segments of the uteroplacental arteries, leaving the myometrial segments with an undisturbed vascular anatomy responsive to vasomotor influences. Where there is no proteinuria associated with the pregnancy-induced hypertension or in cases of essential hypertension uncomplicated by preeclampsia-eclampsia, the sit-

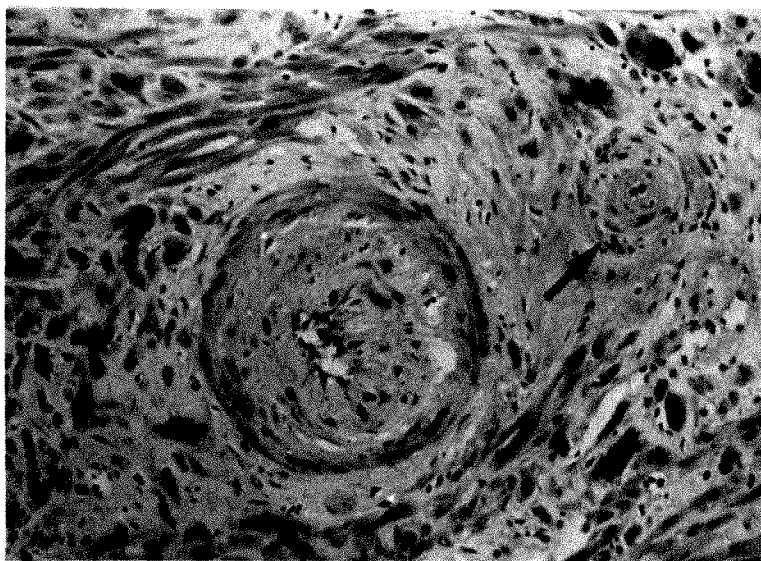


Fig. 10. Preeclampsia: myometrial segment of spiral artery in the placental bed as indicated by the interstitial migratory trophoblast, including giant cells. There are no physiologic changes and the apparent stenosis of the vessel is artifactual and probably due to vascular spasm and processing effects. A small basal artery is shown (arrow). (Hematoxylin and eosin. Original magnification $\times 200$.)

uation is less clear.^{31, 39} Absence of physiologic changes in the myometrial segments of the uteroplacental (spiral) arteries should be diagnosed in such arteries only when there is plentiful nonvillous trophoblast, placental bed giant cells, in their immediate vicinity (Fig. 10). The biopsy may have come from the marginal zone of the placental bed and therefore will contain little if any extravillous trophoblast in the myometrium. Similarly, the smaller basal or straight arteries, $<120\ \mu\text{m}$ in external diameter, are not involved with physiologic changes (Fig. 10), even in normal pregnancy, as they take no part in supplying the placenta.

Arterial lesions. In 1945, Hertig³⁶ first described a distinctive necrotizing lesion of the uterine spiral arteries accompanied by foam cell infiltrates (Fig. 11) in the hypertensive disorders of pregnancy, which he subsequently called acute atherosclerosis.³⁷ Since that time there have been several studies^{15, 16, 20, 23} of this arterial lesion, principally in relation to preeclampsia-eclampsia, and it is thought by many, but not by all, to be virtually pathognomonic of albuminuric hypertension in pregnancy, whether in preeclampsia-eclampsia arising de novo or in preeclampsia-eclampsia superimposed on essential hypertension, renal disease, diabetes mellitus,²⁹ or systemic lupus erythematosus.²⁸ Interestingly, the best material to use to identify acute atherosclerosis is the decidua vera or parietalis and not the placental bed or the basal plate of the placenta. The decidua vera obviously is devoid of migratory trophoblast and its associated effects on maternal tissues, including spiral arteries. The vasculopathy is therefore seen in the decidua vera in its "pure" form, uncomplicated by preex-

isting trophoblast-induced vascular changes. Thus, in studies concerned mainly with vasculopathies, it is good practice to take biopsy specimens of the decidua parietalis in addition to those from the placental bed. Rolled-up strips of the placental membranes³⁸ are a valuable additional source of decidua vera containing portions of decidual spiral arteries, which may show acute atherosclerosis.

Difficulties in interpretation with regard to pathologic lesions arise in the uteroplacental arteries themselves. In their decidual segments the vessels have been altered almost beyond recognition through involvement with endovascular trophoblast and it becomes very difficult to differentiate the characteristic fibrinoid material in the vessel wall from the fibrinoid necrosis of an acute vasculopathy. Furthermore, senescent cells in the physiologic changes, including trophoblast, accumulate lipid in their cytoplasm.²⁵ Nonetheless a good case has been made²¹ that enhanced atheroma-like changes occur in the uteroplacental arteries in cases of severe intrauterine growth retardation independent of the level of the blood pressure. There seems little doubt also that acute necrotizing lesions in small superficial vessels in the basal decidua, but not in the myometrium or decidua vera, can be found in cases of intrauterine growth retardation unassociated with hypertension. What relation these lesions have to the acute atherosclerosis characteristic of preeclampsia-eclampsia remains to be determined. In albuminuric preeclampsia-eclampsia acute atherosclerosis is characteristically found in decidual and myometrial vessels, including basal arteries, that have not been subject to physiologic changes.

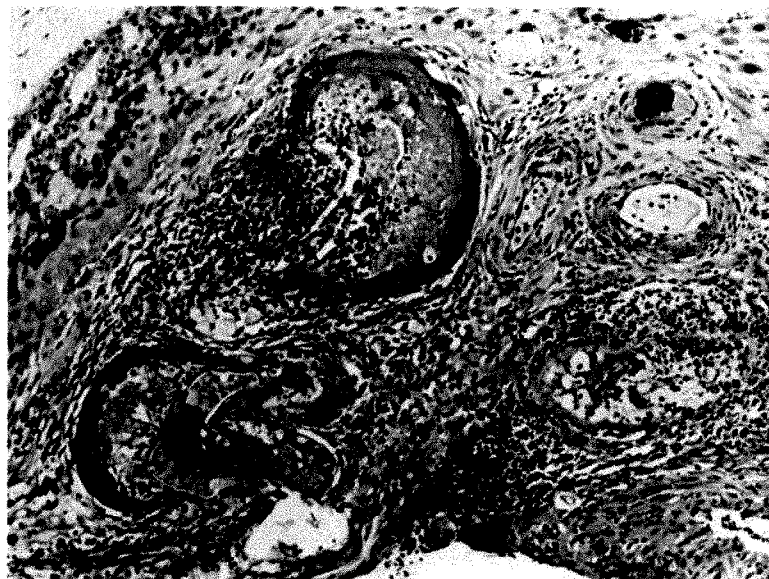


Fig. 11. Typical example of acute atherosclerosis in a spiral artery in a fragment of decidua attached to the placental membranes. The wall of the artery has undergone complete fibrinoid necrosis and there are numerous foam cells plus an extensive perivascular round cell infiltrate. (Hematoxylin and eosin. Original magnification $\times 125$.)

In pregnancies complicated by long-standing hypertension, whether of the essential type or secondary to renal disease, the uteroplacental arteries may show hyperplastic changes added to the physiologic changes.^{16, 21, 31, 39} When preeclampsia-eclampsia is superimposed on preexisting essential hypertension, hyperplastic arteriosclerotic lesions of a surprising degree of severity may be found in the myometrial segments of the uteroplacental arteries uninvolved with physiologic changes to which may be added acute atherosclerosis³⁹ (Fig. 12).

It is not surprising that this spectrum of vasculopathies may lead to vascular thromboses and to vessel rupture with hemorrhage accounting for the high incidence of placental infarction, abruptio placentae, and retroplacental hemorrhage encountered in the hypertensive disorders of pregnancy. It should be borne in mind, however, that these third-trimester events, however dramatic and life-threatening to the fetus and occasionally to the mother they may be, in a sense are epiphenomena. Of more fundamental importance is the appreciation that in many disorders of pregnancy, such as preeclampsia-eclampsia and intrauterine growth retardation, and probably in antepartum hemorrhage and others, the stage is set during the establishment and development of placentation in the first and second trimesters.

Immunologic aspects of the placental bed. The rapid development of immunohistologic technology⁵⁰ over the last decade inevitably has led those interested in the immunologic aspects of normal and abnormal

pregnancy to apply the techniques to studies of the human placenta and, more recently, to the placental bed in early and late pregnancy.^{12-52, 60-62} Published studies can be categorized into four main groups, the first being the detection of the secretory products, pregnancy-specific proteins and hormones, of extravillous trophoblast^{13-15, 52, 61}; the second, the expression of distinctive, possibly unique, trophoblast antigens^{12, 48, 49, 51, 61}; the third, the expression of common biologic antigens, such as the HLA system, by trophoblast elements¹⁶⁻¹⁸; and the fourth, the maternal antibody response to pregnancy.⁶⁰

In general terms it has been found that extravillous trophoblast, both interstitial and endovascular, appears to produce human placental lactogen but not human chorionic gonadotropin.^{13, 11, 62} Class I major histocompatibility complex antigens are expressed by some, but not all, populations of extravillous trophoblast while these antigens are detected on villous trophoblast only at certain stages of pregnancy.^{16-18, 61} Furthermore, the pattern of expression would not appear to be different in pregnancies complicated by preeclampsia.¹⁶ Antibodies derived from trophoblastic tissue and claimed to be specific for trophoblast antigens have been used for the distinction of fetal from maternal tissues where the two are admixed.^{12, 18, 61} In practice, it has been found that there is considerable cross-reaction of these supposed trophoblast-specific antibodies with maternal tissue such as decidua and maternal uterine epithelium,¹⁸ leaving the investigator still dependent on making the distinction between fetal and maternal cells on



Fig. 12. Essential hypertension with preeclampsia: The myometrial segments of a spiral artery show marked hyperplastic changes related to the preexisting hypertension and the largest segment shows superimposed acute atherosclerosis due to the preeclampsia. (Hematoxylin and eosin. Original magnification $\times 125$.)

purely morphologic characteristics. The long-recognized feature of leukocyte infiltrates in the placental bed, often related to endometrial glands, has been shown to consist mainly of macrophages and T lymphocytes,⁶⁰ suggesting that the endometrial glands are subjected to a local immune attack during pregnancy.

The conflicting published findings from immunohistologic investigations of the placental bed in normal and abnormal placentation clearly indicate the need for further and more refined studies of this tissue.⁵⁹

Unanswered questions

It will be obvious from the foregoing that there is still much to be learned, even at the morphologic level, about human placentation, its aberrations, and its response to pathologic influences. Of prime importance is the elucidation of the promotional and controlling factors mediating the behavior of migratory extravillous trophoblast, a characteristic feature of all species with hemochorial placentation,^{10, 63} and this must include the part played by maternal tissues such as decidua. What is the purpose of the flooding of the basal decidua and subjacent myometrium by extravillous trophoblast, other than a possible priming action on the spiral arteries in the placental bed³⁷? Is there a special subset of this trophoblast programmed to attack, open up, and colonize the maternal spiral arteries to convert them to uteroplacental arteries? How does this trophoblast effect the physiologic vascular changes without precipitating thromboses and other vascular catastro-

phies and what is the effect of the extensive and complex alterations to the vessel walls on their autonomic nerve supply⁶⁴⁻⁶⁶?

Underlying these questions and many others is the major conundrum of the mutual immunologic tolerance between two genetically dissimilar tissues, maternal and fetal, a harmony that may be upset in disorders such as spontaneous abortion,⁶⁷⁻⁶⁹ preeclampsia,⁷⁰ and no doubt others.

The hypertensive disorders and intrauterine fetal growth retardation also require more detailed studies at the morphologic level to substantiate or refute the claims that both are associated with defective placentation. Are there two distinct waves of endovascular trophoblast migration to completely convert the spiral arteries in the placental bed to uteroplacental arteries and is the mechanism defective in either or both categories of pregnancy disorder? For that matter it is not even known how many spiral arteries are converted to uteroplacental arteries and how many not in these disorders. The evidence is convincing that well over 100 uteroplacental arteries are formed in normal pregnancy,^{2, 3} and recent work⁷¹ indicates that virtually all spiral arteries in the path of migratory extravillous trophoblast are converted to uteroplacental arteries whereas in preeclampsia-eclampsia and in intrauterine growth retardation a significant proportion escape from or resist involvement with endovascular trophoblast migration.

The vasculopathies of preeclampsia-eclampsia and of

intrauterine growth retardation, about which much has been written and elaborated,^{23, 31, 39, 59, 72} urgently require elucidation as to their pathogenesis and significance. There is no reason to suppose that there is only one specific vascular lesion as it is universally recognized that different pathogenetic mechanisms can result in virtually identical lesions as seen under the microscope. Morphologic studies, while they are an indispensable part of certain types of research activity, may actually mislead the unwary and uncritical investigator to wrong conclusions. On the other hand, it is difficult to see how further progress can be made in the field of human placentation and its associated disorders without a secure morphologic base.

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Tubuloreticular inclusions in placental chorionic villi of rhesus monkeys after maternal treatment with interferon

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Tubuloreticular inclusions were observed in placental chorionic villi of rhesus monkeys after pregnant female monkeys were injected intramuscularly with recombinant leukocyte A interferon (25×10^6 units/kg). They were identified in endothelial cells, fibroblasts, and Hofbauer cells of the chorionic villi, providing evidence that interferon crossed at least part of the maternal-fetal partition. Induction of tubuloreticular inclusions in these cells by exogenous interferon has not been previously reported. Tightly packed regular tubular arrangements appeared in endothelial and Hofbauer cells and loosely organized tubular arrays in fibroblasts. The maximum dimension of the tubuloreticular inclusions measured $3 \mu\text{m}$ and the diameter of the tubules was 20 nm. The tubuloreticular inclusions were continuous with, and surrounded by, smooth endoplasmic reticulum that was often connected to rough endoplasmic reticulum. The tubuloreticular inclusions were not detected in placental chorionic villi from monkeys not treated with interferon or from interferon-treated monkeys 30 days after cessation of treatment. These results indicate that the formation of tubuloreticular inclusions in rhesus monkey placentas was a transient response associated with elevated serum levels of interferon. (AM J OBSTET GYNECOL 1986;155:413-24.)

Key words: Tubuloreticular inclusions, placental chorionic villi, maternal interferon treatment

Cytoplasmic inclusions consisting of tubules arranged in loosely organized configurations or in tightly packed regular patterns have been observed in various pathologic conditions (For reviews see references 1, 2, and 3). The tubules are 20 to 28 nm in diameter and are associated with the endoplasmic reticulum. These aggregates, currently termed tubuloreticular inclusions, have been referred to by several other names,² such as crystalloids or tubular arrays.³ An association between tubuloreticular inclusions and interferon was indicated by the appearance of tubuloreticular inclusions in human lymphoblastoid cell cultures⁴ and chimpanzee lymphoid cell cultures⁵ exposed to interferon and in mononuclear cells from patients treated with interferon.⁶ Inclusions were often found in endothelial cells and lymphocytes from patients with systemic lupus erythematosus⁷ or with acquired immune deficiency syndrome,⁸ both of which are diseases in which serum interferon levels are elevated.⁸ Tubuloreticular inclusions were also reported in endothelial cells and lymphocytes of patients with the acute form of Kaposi's sarcoma⁹; patients with this disease may also have high

serum levels of interferon.⁹ In the present experiment, the observation of tubuloreticular inclusions in placental chorionic villi of pregnant rhesus monkeys treated with recombinant leukocyte A interferon and the failure to detect tubuloreticular inclusions in villi of monkeys not treated with interferon provide new evidence that interferon can initiate their formation.

Material and methods

At the California Primate Research Center, Davis, California, six rhesus monkeys (*Macaca mulatta*) received daily intramuscular injections of recombinant leukocyte A interferon (25×10^6 units/kg) beginning on 79 ± 2 days of gestation. Four monkeys were treated for 21 days; placental tissues were then obtained from these monkeys by hysterotomy. One monkey aborted after 7 days and one aborted after 8 days of treatment. These two monkeys and two of the other four monkeys had previous histories of abortions. Placental tissues were obtained by hysterotomy from six control monkeys (not treated with interferon) from the California Primate Research Center, Davis, California, on day 95 to 100 of gestation. Specimens were fixed in 2.5% phosphate-buffered glutaraldehyde and shipped to Hoffmann-La Roche Inc., Nutley, New Jersey. All specimens were postfixed in 1% phosphate-buffered osmic acid, dehydrated, and embedded in Epon. Semi-thin Epon sections were stained with methylene blue—

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azure II to determine location within the placenta. Ultrathin sections from 19 blocks of placental chorionic villi from treated monkeys and 13 blocks from untreated monkeys were stained with uranyl acetate and lead citrate and examined in a Philips 300 electron microscope.

Results

Tubuloreticular inclusions were observed in samples of chorionic villi from the six placentas of rhesus monkeys treated with interferon. They appeared in all of the 15 blocks examined from five placentas and in one of the four blocks examined from one placenta. Tubuloreticular inclusions were not found in any of the 13 blocks of placenta from the six monkeys not treated with interferon. Structures associated with the placental chorionic villus, including syncytiotrophoblast (S), cytotrophoblast (C), stromal fibroblasts (F), Hofbauer cells (H), and endothelial cells (E) are shown in Figs. 1 to 11a. A dividing endothelial cell is shown in Fig. 3. Tubuloreticular inclusions were identified in endothelial cells, Hofbauer cells, and fibroblasts of the chorionic villi and in a histiocyte-like cell in the cytotrophoblastic shell of the basal plate. They were encountered with greatest frequency in endothelial cells and usually found in 5% to 20% of capillary sections. Tubuloreticular inclusions were occasionally seen in fibroblasts in five blocks from four placentas and in Hofbauer cells in three blocks from two placentas. Tubuloreticular inclusions shown in Figs. 1a, 2a, 2b, 3a, 7a, 7c, and 9a are higher magnifications of tubuloreticular inclusions (arrows) present in endothelial cells in Figs. 1, 2, 3, 7, and 9, respectively. Higher magnifications of tubuloreticular inclusions in fibroblasts (Figs. 7 and 11), in a Hofbauer cell (Fig. 10), and in a histiocyte-like cell (Fig. 8) appear in Figs. 7b, 11a, 10a, and 8a, respectively.

In endothelial cells, tubuloreticular inclusions were arranged in tightly packed arrays. Occasionally, two foci of tubuloreticular inclusions were present in a single transverse section through the capillary endothelium, each located in a different endothelial cell (Fig. 2). The maximum dimension of tubuloreticular inclusions measured 3.0 μm ; inclusions that were tangentially sectioned may have resulted in smaller profiles. Foci of tubuloreticular inclusions displayed branching or irregular contours. They consisted of one or more bundles of tubules arranged in parallel rows; each tubule was approximately 20 nm in diameter. The bundles were oriented at various angles to one another. Since the images observed depend on the plane of the section through the bundle, different images were noted in the same inclusion as the section passed through several bundles (Figs. 1a, 2a, 2b, 4, and 6). In transverse sections (Figs. 2b, center, 4, top, 5, 6, top, and 7c, left), the

tubules appeared round with an outer membrane surrounding a core of low electron-density. In some sections, narrow, dense connections (arrows in Figs. 2a, right, 2b, left, 6, and 7c) appeared between the tubules. Tubules in alternating rows were situated in an intermediate position in relation to the tubules in the preceding and succeeding rows, resulting in a geometric pattern in transverse sections.

Since the thickness of a section was at least twice the diameter of the tubule, each longitudinal section through a bundle of tubules included two or more rows of tubules. The lack of tubule alignment and the connections between the tubules may account for the overall density and striated appearance of longitudinal sections (Figs. 2b, top, and 6, left). Tubules sectioned obliquely exhibited various images, depending on the angle at which the section intersected the bundle of tubules.

Continuity was observed between the tubules and smooth endoplasmic reticulum membranes (arrows in Figs. 5 and 9a), which were often connected to rough endoplasmic reticulum. The cisternae of the endoplasmic reticulum contained moderately electron-dense material. Strands of smooth endoplasmic reticulum also surrounded the tubuloreticular inclusions and extended between the bundles of tubuloreticular inclusions (arrows in Figs. 3a and 7a). Within the tubuloreticular inclusions were tubule-free areas that either were electron lucent (Fig. 2b, center) or contained moderately electron-dense material (Figs. 4, lower left, and 9a).

Most of the endothelial cells in which tubuloreticular inclusions were seen appeared ultrastructurally normal. However, in a few cells, especially those that contained extremely large tubuloreticular inclusions, the cytoplasmic matrix near the inclusions was depleted, the plasma membrane was disrupted (arrow, Fig. 4), and the cytoplasm protruded into the lumen (Fig. 2). Myelin figures (mf, Figs. 1a and 2a) and lysosomes (L, Fig. 7c) were sometimes observed in close association with the inclusions.

In Hofbauer cells, tightly packed, regular arrays of tubuloreticular inclusions, similar to those in endothelial cells, were noted. A dividing Hofbauer cell (H, Fig. 10) containing a tubuloreticular inclusion (arrow) is seen in Fig. 10a. In fibroblasts (F, Figs. 7 and 11), tubuloreticular inclusions were loosely interwoven and irregularly oriented (Figs. 7b and 11a). The degree of irregularity varied among the tubuloreticular inclusions observed and within the individual tubuloreticular inclusion. Tubule diameter, dimension of the tubuloreticular inclusion, and relationship to the endoplasmic reticulum were similar to those of the tightly arranged tubuloreticular inclusions. Except for the presence of

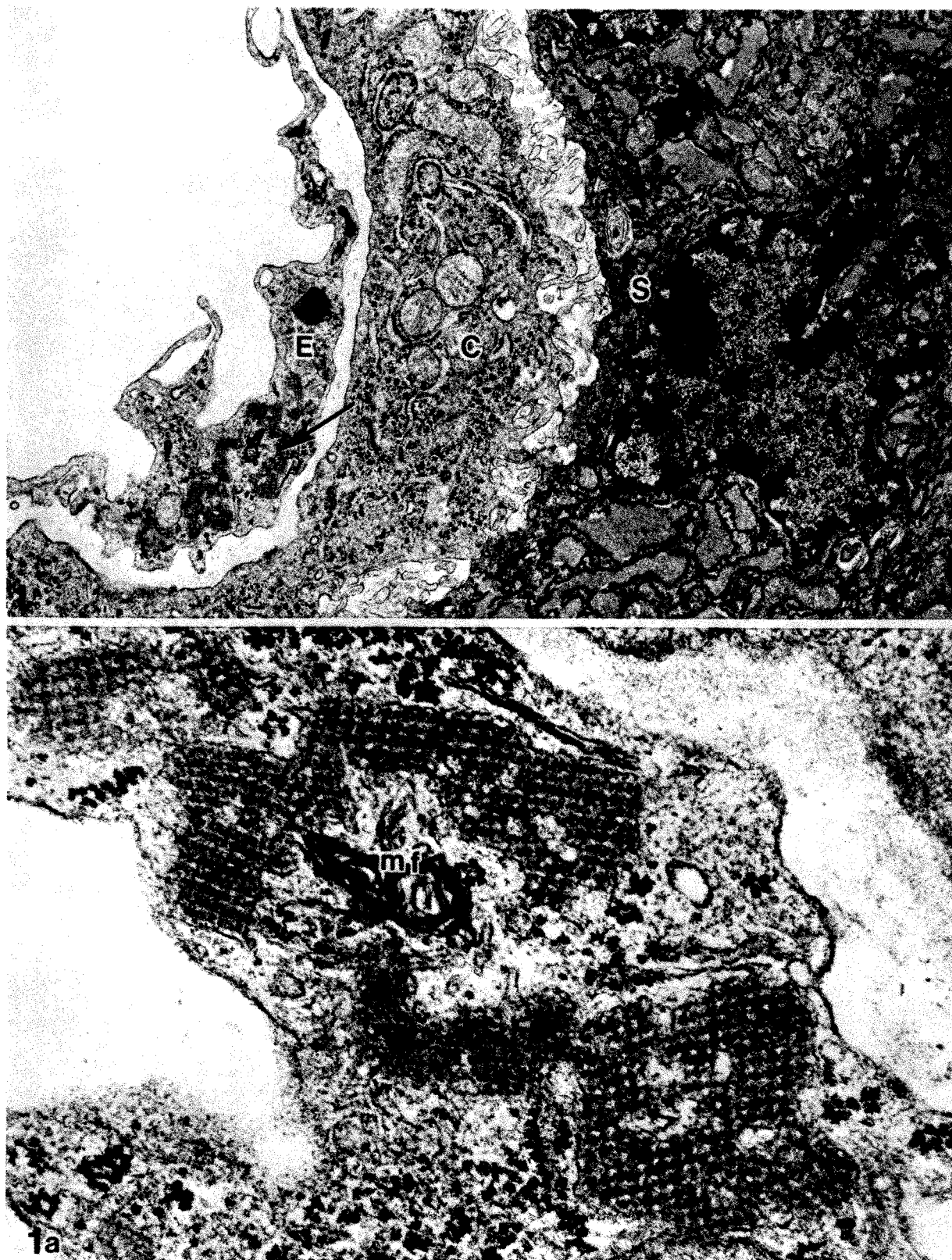


Fig. 1. Portion of chorionic villus showing endothelial cells (*E*), syncytiotrophoblast (*S*), cytotrophoblast (*C*), and tubuloreticular inclusions (*arrow*). (Original magnification $\times 15,800$.)

Fig. 1a. Higher magnification of tubuloreticular inclusion in Fig. 1. Myelin figures (*mf*) are shown. (Original magnification $\times 86,250$.)



Fig. 2. Portion of chorionic villus showing endothelial cells (*E*), syncytiotrophoblast (*S*), lysosome (*L*), and tubuloreticular inclusions (arrows, *a* and *b*). (Original magnification $\times 13,500$.)

Fig. 2a. Higher magnification of tubuloreticular inclusion in Fig. 2 (*a*). Myelin figures (*mf*) and connection between tubules (arrow) are shown. (Original magnification $\times 86,250$.)

Fig. 2b. Higher magnification of tubuloreticular inclusions in Fig. 2 (*b*). Connection between tubules (arrow) is shown. (Original magnification $\times 86,250$.)

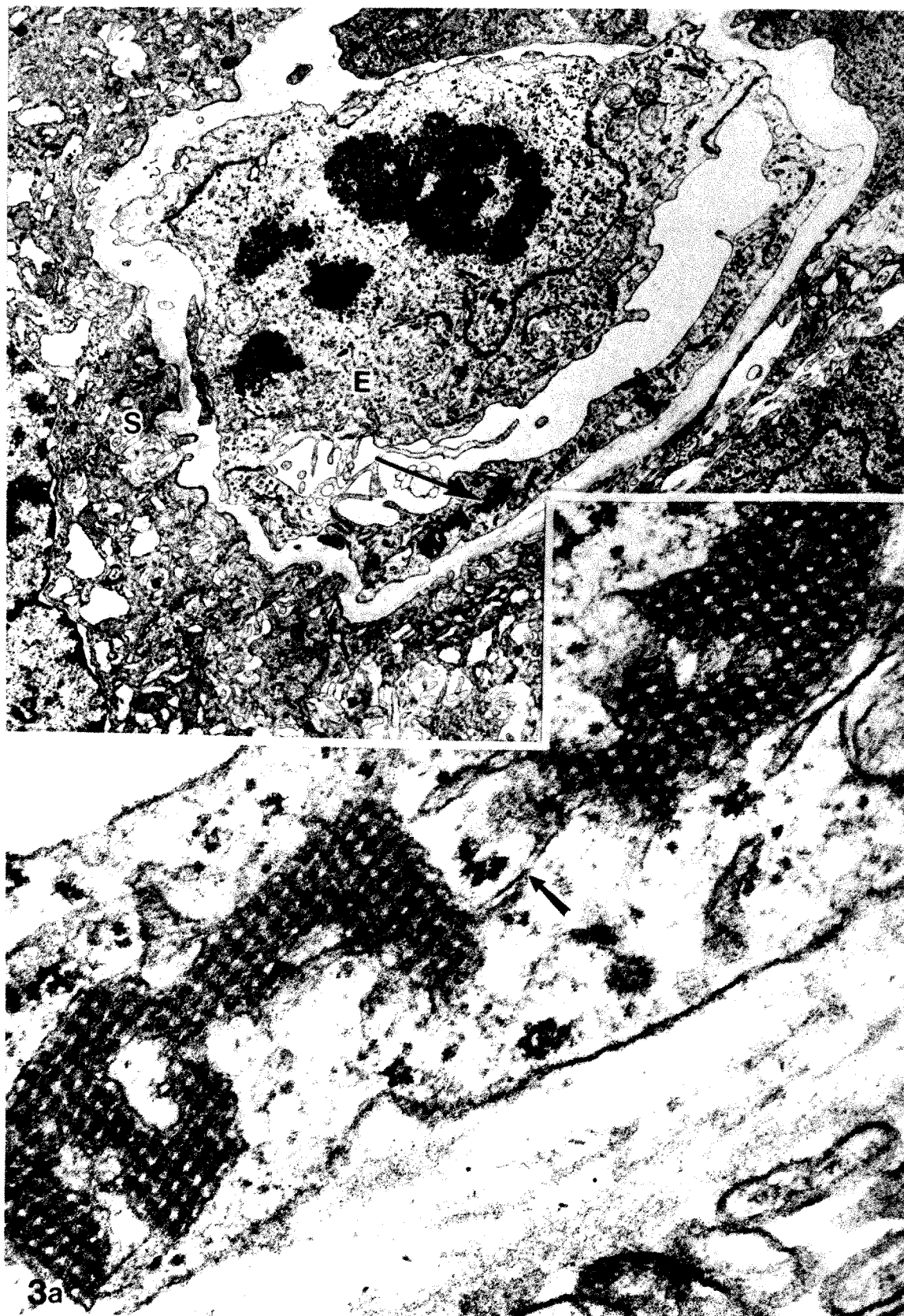


Fig. 3. Portion of chorionic villus showing endothelial cells (*E*), syncytiotrophoblast (*S*), and tubuloreticular inclusion (*arrow*). (Original magnification $\times 13,500$.)

Fig. 3a. Higher magnification of tubuloreticular inclusions in Fig. 3. Smooth endoplasmic reticulum joining bundles of tubuloreticular inclusions (*arrow*) is shown. (Original magnification $\times 113,250$.)

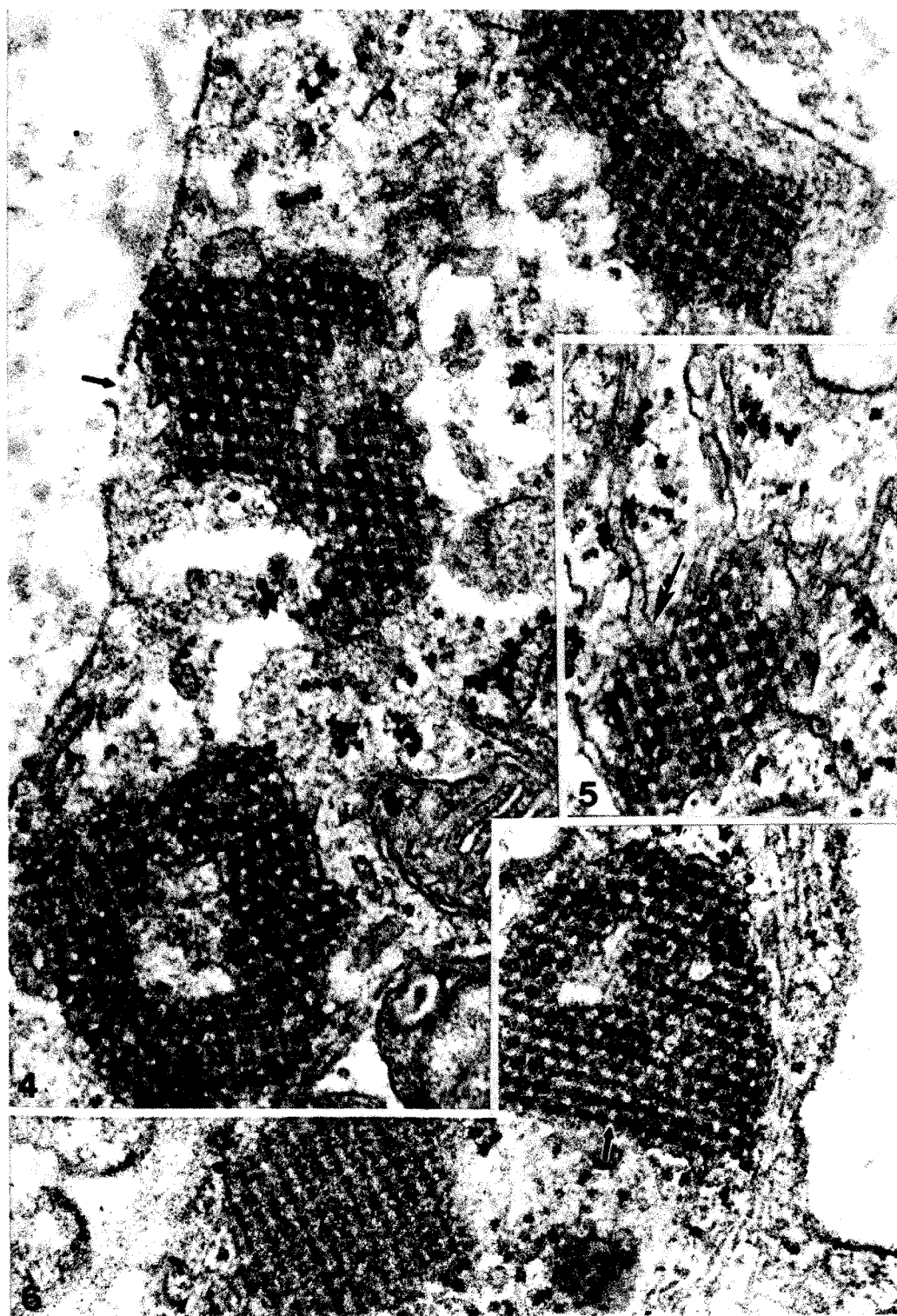


Fig. 4. Tubuloreticular inclusions in endothelial cells. Transversely (*top*) and obliquely sectioned tubuloreticular inclusions and interrupted plasma membrane (*arrow*) are shown. (Original magnification $\times 113,200$.)

Fig. 5. Tubuloreticular inclusions in endothelial cells. Transversely sectioned tubuloreticular inclusions and continuity of tubuloreticular inclusions with smooth endoplasmic reticulum (*arrow*) are shown. (Original magnification $\times 113,200$.)

Fig. 6. Tubuloreticular inclusions in endothelial cells. Transversely (*top*) and longitudinally (*bottom*) sectioned tubuloreticular inclusions and connection between tubules (*arrow*) are shown. (Original magnification $\times 113,200$.)

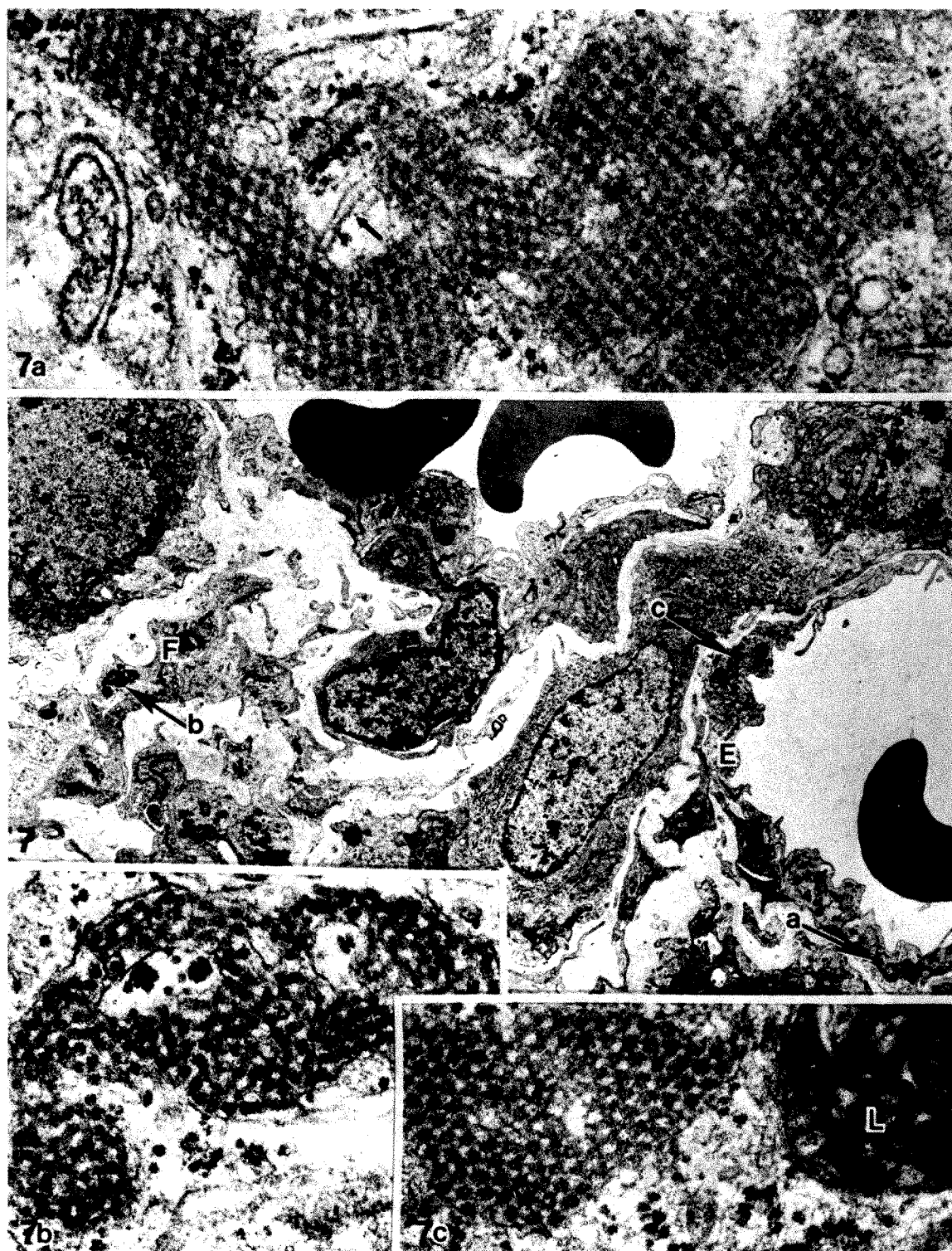


Fig. 7. Portion of chorionic villus showing endothelial cell (E), fibroblast (F), and tubuloreticular inclusions (arrows, a, b, and c). (Original magnification $\times 6480$.)

Fig. 7a. Higher magnification of tubuloreticular inclusions in Fig. 7 (a). Smooth endoplasmic reticulum joining bundles of tubuloreticular inclusions (arrows) is shown. (Original magnification $\times 103,800$.)

Fig. 7b. Higher magnification of tubuloreticular inclusions in Fig. 7 (b). (Original magnification $\times 86,250$.)

Fig. 7c. Higher magnification of tubuloreticular inclusions in Fig. 7 (c). Lysosome (L) and connections between tubules (arrows) are shown. (Original magnification $\times 103,800$.)

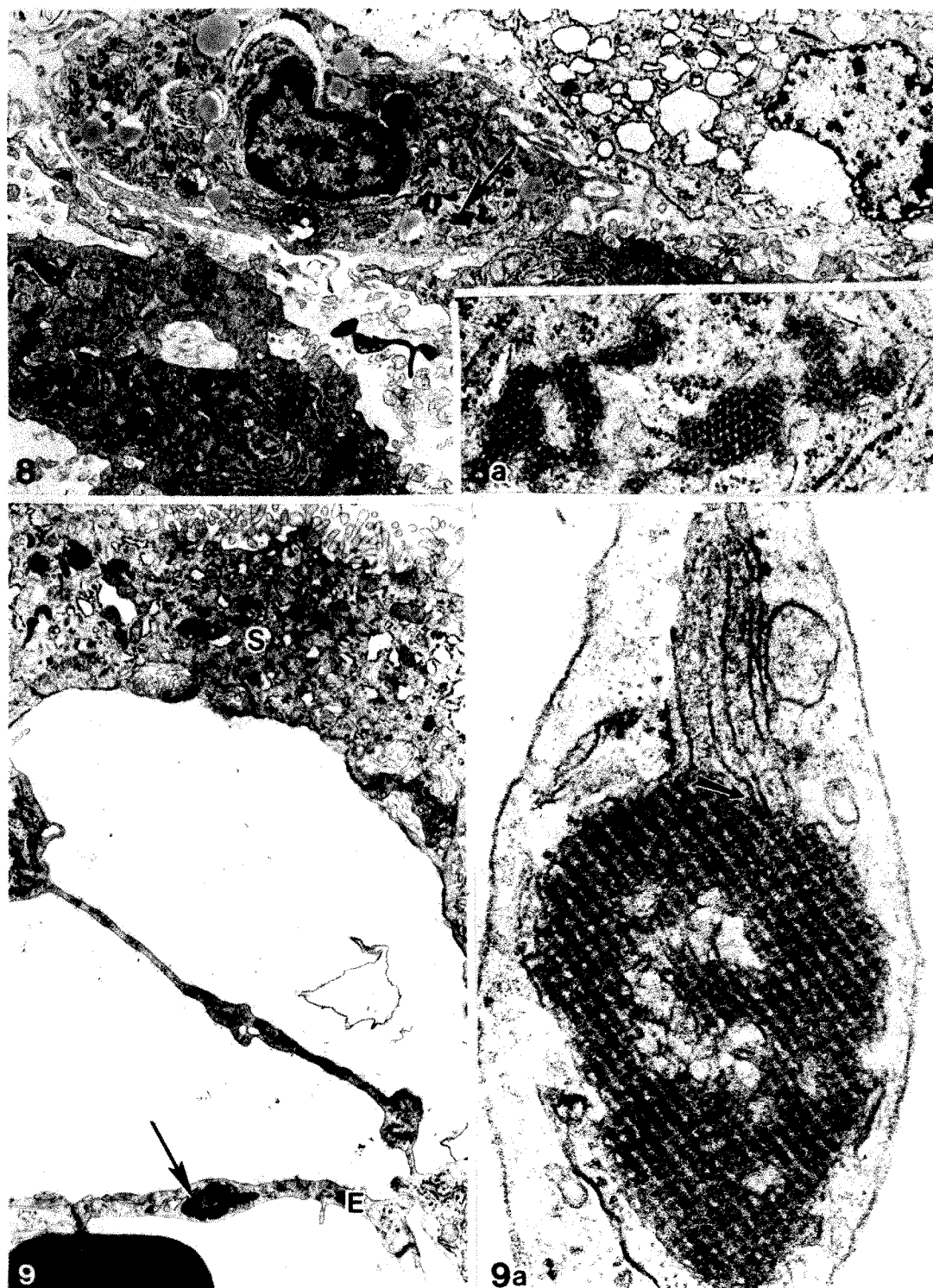


Fig. 8. Part of the cytotrophoblastic shell containing a histiocyte-like cell (*top, left*) that contains tubuloreticular inclusions (*arrow*). (Original magnification $\times 8630$.)

Fig. 8a. Higher magnification of tubuloreticular inclusions in Fig. 8. (Original magnification $\times 49,135$.)

Fig. 9. Chorionic villus syncytiotrophoblast (*S*) and endothelial cell (*E*) containing tubuloreticular inclusions (*arrow*). (Original magnification $\times 10,800$.)

Fig. 9a. Higher magnification of tubuloreticular inclusion in Fig. 9. Continuity between the smooth endoplasmic reticulum and tubuloreticular inclusion is indicated (*arrow*). (Original magnification $\times 113,250$.)

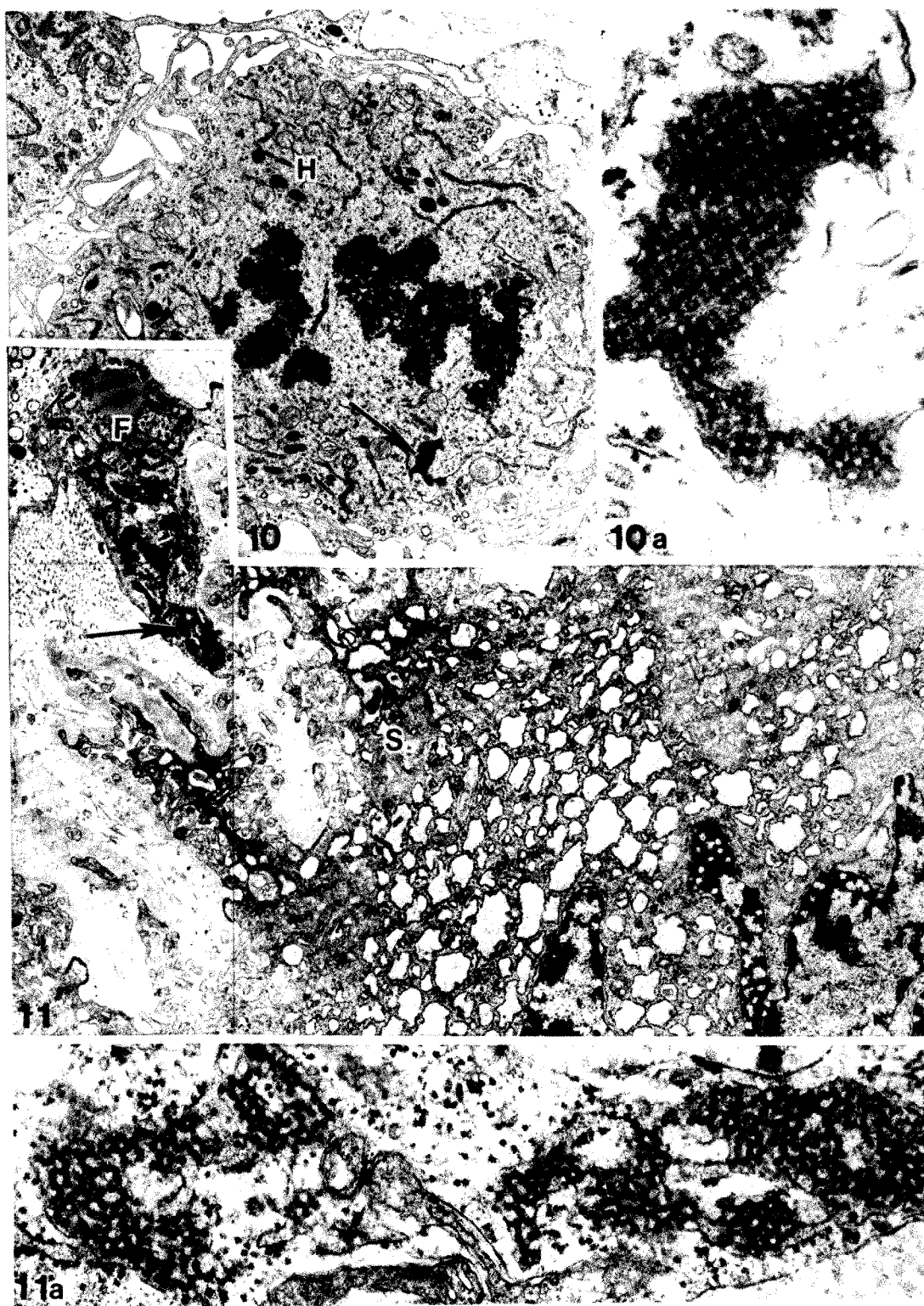


Fig. 10. A dividing Hofbauer cell (*H*) contains tubuloreticular inclusions (*arrow*). (Original magnification $\times 8640$.)

Fig. 10a. Higher magnification of tubuloreticular inclusions in Fig. 10. (Original magnification $\times 86,250$.)

Fig. 11. Syncytiotrophoblast (*S*) and fibroblast (*F*) containing tubuloreticular inclusions (*arrow*) that are loosely organized. (Original magnification $\times 10,800$.)

Fig. 11a. Higher magnification of loosely organized tubuloreticular inclusions in Fig. 11. (Original magnification $\times 59,000$.)

tubuloreticular inclusions, the fibroblasts were morphologically normal.

Comment

Tubuloreticular inclusions were discovered in cells of placental chorionic villi during the investigation of possible effects of interferon on C-type virus particles in the rhesus monkey placenta. The ability of interferon to inhibit normal replication of C-type virus particles *in vitro* plus the consistent appearance of C-type particles in placental chorionic villi of several primate species provided the rationale for initiating this study. During the examination of placentas from interferon-treated rhesus monkeys, tubuloreticular inclusions were found in endothelial cells, fibroblasts, and Hofbauer cells of the chorionic villi. Tubuloreticular inclusions were not observed in chorionic villi from rhesus monkeys that did not receive interferon, indicating a relationship between the administration of interferon and the production of tubuloreticular inclusions. The induction of tubuloreticular inclusions in endothelium, fibroblasts, and Hofbauer cells by exogenous interferon has not been previously reported.

Another experiment in which tubuloreticular inclusions were observed in placental chorionic villi endothelial cells involved humans infected with rubella virus.¹⁰ Tubuloreticular inclusions were also present in endothelial cells of various tissues from the infected fetuses but not in cells from control fetuses obtained through legal abortions for psychiatric reasons. Tissues from infants with congenital rubella syndrome also contained tubuloreticular inclusions.¹¹ Interferon was induced in placental and fetal cell cultures infected with rubella virus.¹² Thus, tubuloreticular inclusions in chorionic villi endothelium in placentas obtained from mothers infected with rubella virus as well as in the infected fetuses and congenitally infected infant may be the result of endogenous interferon production induced by rubella virus.

The preferential, but not exclusive, formation of tubuloreticular inclusions in endothelial cells in the current experiment is poorly understood. Endothelial cells are considered biologically active with specialized functions, depending on their location within the organism. It is possible that tubuloreticular inclusions represent the product of endothelial cells that have been stimulated to process or catabolize a particular substrate such as interferon. In addition, the extensive exposure of endothelial cells to circulating proteins may make them a privileged reaction site.

The formation of tubuloreticular inclusions in cells of the placental villus core was presumably initiated by either interferon itself or an intermediate product, induced or activated by interferon. However, in order to reach fibroblasts and Hofbauer cells from the maternal

circulation, this initiator must cross the trophoblast, the trophoblast basement membrane, and the stromal matrix. In addition to the above, passage through the endothelial basement membrane would be necessary in order to reach the capillary endothelium, which acts as the final regulator of substances transported from the maternal to the fetal circulation. The ability of interferon to cross this placental partition has been controversial and may vary with species. Investigators have reported that the placenta inhibits the free passage of interferon into the fetal circulation of sheep, mice, and rabbits.¹³ In the latter study, a significant concentration of endogenous virus-induced interferon was found in the placentas of pregnant rabbits, but only a negligible amount was detected in fetal tissues. However, the placenta's permeability to interferon in the rat on the nineteenth day of gestation has been demonstrated. In humans, tubuloreticular inclusions appeared in lymphocytes obtained from umbilical cord blood from infants of two patients with systemic lupus erythematosus,¹⁴ a disease associated with elevated levels of endogenous serum interferon. The presence of tubuloreticular inclusions in these human neonates indirectly supports the concept of interferon's transplacental passage in humans. The presence of tubuloreticular inclusions in endothelial cells of the placental villus of rhesus monkeys treated with interferon in this experiment is highly suggestive but inconclusive evidence that interferon entered the fetal circulation.

Tubuloreticular inclusions have been previously reported in tissues from clinically ill as well as overtly normal rhesus monkeys. They were present in endothelial and spindle cells of tumors induced in rhesus monkeys by chicken sarcoma virus and in leukemic leukocytes, endothelial cells of lymph nodes, and Kupffer cells of livers from rhesus monkeys that developed leukemia following exposure to whole-body proton irradiation. Crystalline bodies resembling tubuloreticular inclusions were reported in glomerular capillary endothelium, endothelium of ocular blood vessels, pulmonary capillary endothelium, and hepatic sinusoidal and portal vessel endothelium from presumed normal rhesus monkeys. Tubuloreticular inclusions were also found in glomerular and thymic endothelial cells and splenic lymphoid cells of rhesus monkey fetuses from untreated mothers (unpublished observations). Since tubuloreticular inclusions have been observed in a wide variety of conditions, the implications of their presence in these rhesus monkey tissues are uncertain. Tightly packed tubuloreticular inclusions are often associated with diseases of known or suspected viral etiology.³ Latent viruses often exist in clinically normal rhesus monkeys and the tubuloreticular inclusions may reflect low titers of circulating interferon in response to a latent virus infection. Therefore, the reports of tubuloretic-

ular inclusions in "normal" animals should be carefully evaluated. The description of tubuloreticular inclusions in a few reports does not warrant the assumption that tubuloreticular inclusions are a consistent cytoplasmic component of rhesus monkey endothelial cells. In addition, the failure to detect tubuloreticular inclusions in placentas of monkeys not treated with interferon also precludes such an assumption.

In regard to the presence of tubuloreticular inclusions in rhesus monkey fetal tissues, it is possible that tubuloreticular inclusions indicate elevated titers of interferon in the developing fetus. Production of interferon by virus-infected human amniotic membranes has been reported¹⁵ and interferon has been detected in the amniotic fluid but not in the sera of pregnant women.¹⁵ Since proteins from amniotic fluid swallowed by the rhesus monkey fetus have been demonstrated in the intestine of the fetus, any interferon present in the fetus also could have been derived from the amniotic fluid. The possible role of interferon in cell differentiation and regulation of cell replication in the developing fetus has been suggested. The effect of interferon on granulocytic differentiation and on protein, ribonucleic acid, and deoxyribonucleic acid synthesis suggests that it can affect fundamental cellular processes and functions. In addition to protecting the fetus from viral infections, interferon may also be involved in the protection of the fetal allograft because of its depressive effect on graft versus host reactions.

The tubuloreticular inclusions observed in this experiment and those previously described have certain features in common. They are similar in their total dimensions, tubule diameter, continuity with and enclosure by the endoplasmic reticulum, and predilection for endothelial cells. Both tightly packed arrangements and loose arrays appeared in the same specimen in this and other studies.¹⁶ A transition between the two forms¹⁶ and a relationship between the packing density of the tubuloreticular inclusions and the variation in its appearance have been suggested. The presence of tubuloreticular inclusions in dividing cells in the present and other studies¹ can be regarded as evidence against a cytotoxic effect.

Cytochemical investigations of the composition of tubuloreticular inclusions have demonstrated their digestion by Pronase and pepsin and their insensitivity to trypsin and ribonuclease.² Based on these findings, it was concluded that the inclusions are composed of acidic-type proteins and probably do not contain viral nucleoproteins.² Subsequent studies suggested the presence of phospholipid and acidic glycoprotein⁷ and indicated the requirement for new ribonucleic acid and polypeptide synthesis for their formation.⁴

Tubuloreticular inclusions are probably transitory structures that eventually degenerate after termination

of interferon treatment or when interferon levels are reduced. Although they were consistently observed in placentas from rhesus monkeys treated with interferon until the day of hysterotomy, they were not evident in placentas from monkeys in which interferon treatments were completed 30 days before hysterotomy (unpublished observations). Tubuloreticular inclusions were also no longer detected in human peripheral blood mononuclear cells 5 to 16 days after cessation of interferon treatment.⁶ The close association of lysosomes and myelin figures with tubuloreticular inclusions sometimes observed in this study (Figs. 1a, 2a, and 7c) may signify tubule degeneration.

The significance of tubuloreticular inclusions remains controversial. Suggestions regarding their significance include a cellular response to a wide variety of stimuli, manifestation of a cellular reaction to injury or pathologic or abnormal conditions, a defective virus, a reaction product to autoantibodies, or an indicator of the presence of interferon. Based on (1) the presence of tubuloreticular inclusions in the placentas of interferon-treated monkeys only, (2) reports of their appearance in diseases associated with elevated serum interferon levels and in a diversity of other disorders, (3) their association with the endoplasmic reticulum, and (4) their proteinaceous nature, it is tentatively concluded that the inclusions are a cellular biosynthetic response inducible by interferon and various other agents. The function and nature of this response remain to be determined.

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A complete list of the original 94 references submitted with this article is available on request.

Induction of subcutaneous and intraperitoneal abscesses in mice by *Neisseria gonorrhoeae* and *Bacteroides* species

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The pathogenicity in relation to encapsulation and potential for synergy between *Neisseria gonorrhoeae* and *Bacteroides* spp. was studied by subcutaneous and intraperitoneal inoculation of single organisms or mixtures of these organisms into mice. Ten isolates of *N. gonorrhoeae* and 12 isolates of *Bacteroides* spp. were studied. When injected alone, only heavily encapsulated organisms induced subcutaneous and intraperitoneal abscesses. However, *N. gonorrhoeae* did not induce intraperitoneal abscesses and did not survive in intraperitoneal abscesses even when inoculated with *Bacteroides* spp. Abscesses developed after subcutaneous inoculation of mixtures of heavily encapsulated *Bacteroides* and heavily or slightly encapsulated *N. gonorrhoeae* or of slightly encapsulated *Bacteroides* and heavily encapsulated *N. gonorrhoeae*. Heavily encapsulated *N. gonorrhoeae* did not survive in subcutaneous abscesses longer than 3 days after being inoculated alone and survived no longer than 7 days after inoculation with heavily encapsulated *Bacteroides* spp. An increase in the colony-forming units of *Bacteroides* spp. per abscess was observed when they were inoculated with *N. gonorrhoeae*. When mixed with heavily encapsulated isolates, slightly encapsulated *N. gonorrhoeae* or *Bacteroides* isolates became heavily encapsulated. These data demonstrate the effect of encapsulation on the relationship between *N. gonorrhoeae* and *Bacteroides* spp. and the potential for synergy between these organisms. (AM J OBSTET GYNECOL 1986; 155:424-9.)

Key words: *Neisseria gonorrhoeae*, *Bacteroides* spp., pelvic inflammatory disease, abscess, bacterial synergy

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Recent investigations^{1,4} have documented the polymicrobial etiology of acute salpingitis and pelvic inflammatory disease. Mixed aerobic and anaerobic bacterial flora were recovered from cultures obtained through culdocentesis or laparoscopy. The most frequent pathogens appear to be *Neisseria gonorrhoeae* and anaerobic bacteria (most commonly anaerobic cocci and *Bacteroides* spp.). Recent studies have demonstrated the recovery of *Chlamydia trachomatis* in up to a third of these infections.³ Although *N. gonorrhoeae* is frequently recovered from cervical cultures, it is less commonly recovered from intra-abdominal sites.⁶ In approximately one third of patients with pelvic inflammatory disease only *N. gonorrhoeae* was isolated from intra-abdominal cultures; in another third *N. gonorrhoeae* was recovered and was mixed with other anaerobic and aerobic bacteria, and in the final third only anaerobic and aerobic bacteria were found.

Several theories were proposed to explain the pathogenic role of *N. gonorrhoeae* and its relationship to the other flora. One explanation proposed a direct role of *N. gonorrhoeae* in initiating the infection, causing local inflammatory changes that allow access to the upper genital tract for the other flora.^{1,5} An alternative theory assumed that polymicrobial pelvic inflammatory disease can be initiated without the involvement of *N. gonorrhoeae*.^{2, 1, 6}

In this study an evaluation was made of the relationship between *N. gonorrhoeae* and the *Bacteroides* spp. commonly recovered in salpingitis and pelvic inflammatory disease and the effect of encapsulation on that relationship.

Material and methods

Organisms. All organisms were recent clinical isolates recovered from genital tract infections in women. *N. gonorrhoeae* isolates were recovered from cervical cultures, while all the *Bacteroides* spp. were recovered through culdocentesis or laparoscopy. The organisms included 10 isolates of *N. gonorrhoeae* and four isolates each of *Bacteroides bivius*, *Bacteroides fragilis*, and *Bacteroides melaninogenicus*. The *Bacteroides* spp. were kept frozen in skim milk at -70°C . All *N. gonorrhoeae* were fresh clinical isolates. Strains were identified by standard criteria.^{7, 8} The presence of a capsule was established by the Hiss stain⁷ and confirmed by electron microscopic examination after staining with ruthenium red.⁹ The bacterial isolates were divided into three groups according to the presence and the percentage of encapsulated organisms as follows: (1) nonencapsulated, when no encapsulated organisms were seen among 500 cells on each of two slides; (2) slightly encapsulated, when $<1\%$ of 500 organisms on each of two slides were encapsulated; (3) heavily encapsulated,

when $>50\%$ of the organisms were encapsulated. No encapsulated organisms were present in the range of 1% to 50%. Six of the 10 *N. gonorrhoeae* were heavily encapsulated, three were slightly encapsulated, and one was not encapsulated. Six of the 12 *Bacteroides* spp. (two each of the three *Bacteroides* spp.) were heavily encapsulated, and six (two each of the three *Bacteroides* spp.) were slightly encapsulated.

Animals. All experiments were performed with 6- to 8-week-old female mice (20 to 25 gm) from the albino Swiss mouse colony of the Naval Medical Research Institute (NMRI/NIH-CV). The animals were raised under conventional conditions.

Inoculum preparation. To avoid the gradual loss of capsular material that can occur after several in vitro passages, only fresh cultures were used. *N. gonorrhoeae* were grown on chocolate agar and incubated for 36 to 48 hours at 37°C in 5% carbon dioxide. Frozen *Bacteroides* suspensions were thawed to room temperature, subcultured on supplemented brain-heart infusion agar (BHI-S, Difco Laboratories, Detroit, Michigan),⁸ and incubated for 24 hours at 37°C in an anaerobic glove box.

Infection of mice. Mice were infected by subcutaneous injection into the right groin or intraperitoneally with 0.1 ml volumes of suspensions in saline solution containing 10^9 bacteria per milliliter of each of the bacteria used. An abscess was defined microscopically as a localized encapsulated collection of pus. The mice were put to death by cervical dislocation. The maximal diameter of the abscesses was measured with calipers. For the determination of the total bacterial contents of subcutaneous abscesses, the abscess material was removed aseptically and homogenized in an anaerobic glove box in 1 ml of sterile saline solution in a ground-glass tissue homogenizer. Tenfold serial dilutions of the homogenates were made in sterile saline solution, and 0.1 ml volumes of each dilution were spread in triplicate on brain-heart infusion agar and chocolate agar plates. Colonies were counted following incubation at 37°C for 48 hours in an anaerobic chamber (for anaerobes) or in 5% carbon dioxide (for *N. gonorrhoeae*). The abscesses from two animals in each experimental group were examined histologically after being stained with hematoxylin and eosin.

Experimental design. Experiments usually consisted of 100 to 120 animals tested simultaneously, and each experiment was done twice. The ability of the single organisms or mixtures of two organisms to cause subcutaneous or intraperitoneal abscesses was determined by subcutaneous or intraperitoneal inoculations of pure or mixed cultures of 10^8 of each isolate into groups of 10 animals per bacterial strain or bacterial combination. The animals were observed for 28 days, and the sub-

Table I. Summary of subcutaneous abscess formation by encapsulated and nonencapsulated *Bacteroides* spp. (*B. bivius*, *B. fragilis*, and *B. melaninogenicus*) and *N. gonorrhoeae* injected alone or in combinations

	Organism injected alone		Nonencapsulated <i>N. gonorrhoeae</i> (1 strain)		Slightly encapsulated <i>N. gonorrhoeae</i> (3 strains)		Heavily encapsulated <i>N. gonorrhoeae</i> (6 strains)	
	No.*	%	No.*	%	No.*	%	No.*	%
Organism injected alone								
Subcutaneous	—	—	0/120	0	0/60	0	98/120	82
Intraperitoneal	—	—	0/120	0	0/60	0	0/120	0
Slightly encapsulated <i>Bacteroides</i> spp. (6 strains)								
Subcutaneous	0/120	0	0/120	0	0/360	0	605/720	84
Intraperitoneal	0/120	0	0/120	0	0/360	0	5/720	1
Heavily encapsulated <i>Bacteroides</i> spp. (6 strains)								
Subcutaneous	103/120	86	91/120	76†	568/720	79†	677/720	94
Intraperitoneal	65/120	54	53/120	44†	382/720	53†	490/720	68†

*Number of animals with abscess per number of animals inoculated.

†Highly significantly different ($p < 0.001$) compared with value when injected with slightly encapsulated *Bacteroides* spp.

cutaneous abscess sizes were measured by external measurement of maximal diameter without the animals being put to death. Animals infected intraperitoneally were put to death after 14 days, and semi-quantitative cultures were obtained from the peritoneal cavity and abscess contents. Assessment of the quantitative number of organisms in each subcutaneous abscess induced by single or mixed flora of encapsulated *Bacteroides* spp. and *N. gonorrhoeae* was monitored in a group of 36 mice. Six mice were put to death every other day, starting from the first day after inoculation.

Statistical analysis. Statistical analysis was accomplished with the Student *t* test of independent means.

Results

Abscess formation by single organisms

N. gonorrhoeae. After subcutaneous inoculation into mice of the six heavily encapsulated isolates, abscesses developed in 82% of the mice within 24 hours and reached a maximum diameter of 8 mm within 48 hours (Table I). None of the abscesses drained and the abscesses regressed in size and disappeared spontaneously by day 6. After subcutaneous inoculation of the three slightly encapsulated isolates and the single nonencapsulated isolate, no abscesses developed. No abscesses developed after intraperitoneal inoculation with any of the *N. gonorrhoeae* isolates.

Bacteroides spp. Abscesses developed only after inoculation of the six heavily encapsulated *Bacteroides* isolates. The subcutaneous abscesses were formed within 24 to 48 hours in 86% of the mice and reached a maximal diameter of 18 mm within 5 to 7 days. Up to 90% of the abscesses drained spontaneously at 10 to 14 days, and the area healed within 30 to 35 days. After intraperitoneal inoculation of heavily encapsulated *Bacteroides*, abscesses were seen in 54% of the animals.

Abscesses induced by mixtures of *Bacteroides* and

N. gonorrhoeae. No abscesses were formed after subcutaneous or intraperitoneal inoculation of the three slightly encapsulated and the single nonencapsulated *N. gonorrhoeae* isolates mixed with nonencapsulated *Bacteroides* spp. (Table I). Abscesses were, however, observed after subcutaneous and intraperitoneal inoculation of all three of the heavily encapsulated *Bacteroides* species tested, mixed with all of 10 *N. gonorrhoeae* isolates (heavily or slightly encapsulated or nonencapsulated). Abscesses were frequently observed after subcutaneous inoculation but very rarely after intraperitoneal inoculation of slightly encapsulated *Bacteroides* and heavily encapsulated *N. gonorrhoeae*.

There were, however, differences in the sizes and duration of these abscesses in each of these combinations. No differences were noted in these features among the three *Bacteroides* spp. tested. Abscesses after subcutaneous inoculation were induced by a mixture of heavily encapsulated *Bacteroides* and the single nonencapsulated *N. gonorrhoeae*, lasted 10 to 12 days, and reached a maximal diameter of 18 mm. The *N. gonorrhoeae* strain was never recovered from the abscesses.

Abscesses induced by subcutaneous inoculation of mixtures of encapsulated *Bacteroides* and slightly encapsulated *N. gonorrhoeae* lasted 12 to 18 days and reached a maximal diameter of 24 mm. *N. gonorrhoeae* was isolated from 61% of these abscesses only until the seventh day after inoculation. Encapsulated *N. gonorrhoeae* were recovered only on the fifth and seventh days after inoculation in 69% of the animals infected with two of the three encapsulated *N. gonorrhoeae*.

Abscesses induced by subcutaneous inoculation of combinations of encapsulated *Bacteroides* and heavily encapsulated *N. gonorrhoeae* lasted for 12 to 18 days and reached a maximal diameter of 27 mm. *N. gonorrhoeae*

Table II. Number of *Bacteroides* spp. and *N. gonorrhoeae* (colony-forming units) in subcutaneous abscesses in mice when injected alone or in combination

Organism injected	Days after inoculation*					
	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Abscess induced by one organism						
<i>N. gonorrhoeae</i>	5.1 ± 0.8	3.3 ± 1.2	NG†	NG†	NG†	NG†
<i>B. bivius</i>	6.5 ± 0.7	7.4 ± 0.6	8.7 ± 1.1	8.3 ± 1.4	9.0 ± 1.2	10.4 ± 1.6
<i>B. fragilis</i>	5.0 ± 0.5	7.2 ± 1.1	9.2 ± 1.8	9.5 ± 0.8	9.1 ± 1.3	11.3 ± 1.4
<i>B. melaninogenicus</i>	6.8 ± 0.6	7.8 ± 1.3	8.3 ± 1.0	8.1 ± 0.7	9.4 ± 1.6	10.8 ± 1.0
Abscess induced by two organisms						
<i>N. gonorrhoeae</i> and <i>B. bivius</i>	6.2 ± 0.8	5.4 ± 0.9	3.3 ± 0.4	1.8 ± 0.8	NG	NG
<i>N. gonorrhoeae</i> and <i>B. fragilis</i>	5.7 ± 0.6	8.4 ± 0.4‡	10.2 ± 1.3‡	11.8 ± 1.8‡	11.2 ± 0.6‡	12.4 ± 1.5‡
<i>N. gonorrhoeae</i> and <i>B. melaninogenicus</i>	5.7 ± 0.4	5.5 ± 1.0	3.6 ± 0.7	2.4 ± 0.4	NG	NG
<i>B. bivius</i> and <i>B. fragilis</i>	4.2 ± 1.1	7.7 ± 1.2	9.3 ± 1.3	10.1 ± 2.1	11.5 ± 1.5‡	12.3 ± 1.8‡
<i>B. bivius</i> and <i>B. melaninogenicus</i>	6.5 ± 1.3	4.0 ± 0.9	2.1 ± 0.6	NG	NG	NG
<i>N. gonorrhoeae</i> and <i>B. melaninogenicus</i>	6.1 ± 0.7	8.2 ± 0.2‡	10.3 ± 0.9‡	11.5 ± 1.8‡	10.6 ± 1.6‡	11.8 ± 1.8

*Mice were infected and treated as described in Material and methods. All organisms were heavily encapsulated before inoculation. The colony-forming units are expressed as log 10 per abscess and are the mean ± SD of specimens from six mice in each group.

†NG = No growth.

‡Significant difference ($p < 0.01$) in colony-forming units in abscess induced by combination of *Bacteroides* and *N. gonorrhoeae* as compared with abscess induced by *Bacteroides* alone.

were never recovered from the abscesses after the seventh day.

After subcutaneous inoculation with slightly encapsulated *Bacteroides* and heavily encapsulated *N. gonorrhoeae*, abscesses lasted for 10 to 16 days and reached a maximal size of 18 mm. *N. gonorrhoeae* were recovered only up to the seventh day after inoculation. Three of the six slightly encapsulated *Bacteroides* became heavily encapsulated in 88% of the animals after the ninth day.

In the animals who had intraperitoneal abscesses, the number of abscesses varied from one to six, and their maximal diameter varied from 3 to 14 mm. There was no difference in the size or number of abscesses between the groups. *N. gonorrhoeae* were never recovered in intraperitoneal abscesses, and only the heavily encapsulated *Bacteroides* persisted in those sites. Two of the three slightly encapsulated *N. gonorrhoeae* and three of the six slightly encapsulated *Bacteroides*, which were observed to become heavily encapsulated after subcutaneous inoculation, were also observed to become heavily encapsulated when they were recovered 5 to 7 days after intraperitoneal inoculation in 56% of the animals examined. This phenomenon occurred only in abscesses where the other bacterial isolate was heavily encapsulated.

Pathologic examination. Studies of the abscesses induced by one or two organisms showed them to have a central area of necrotic cells, fibrin, and bacteria, surrounded by a band of leukocytes and a distinct collagen capsule.

Number of organisms (colony-forming units) in subcutaneous abscesses induced by heavily encapsulated isolates. Abscesses were induced by subcuta-

neous inoculation of one heavily encapsulated strain of each of the four bacterial species (one *N. gonorrhoeae* and three *Bacteroides* spp.). After inoculation, abscesses induced by *N. gonorrhoeae* alone reached a peak number of about 10^3 organisms per abscess on day 1 (expressed in colony-forming units) (Table II). A rapid decline occurred by day 3, and no viable organisms were observed by day 5. The number of organisms in abscesses induced by *Bacteroides* spp. alone increased gradually and reached about 10^{10} organisms per abscess by day 11.

After the inoculation of *N. gonorrhoeae* mixed with *Bacteroides* spp., the colony-forming units of *N. gonorrhoeae* gradually decreased until they were not recovered. In contrast, the colony-forming units of all *Bacteroides* spp. were higher than when these organisms were injected alone and reached a peak on day 11. These differences were especially significant in abscesses induced by *B. bivius* and *B. melaninogenicus*.

Comment

The bacterial combinations used in our study often occur in salpingitis and pelvic inflammatory disease. The synergy between *Bacteroides* spp. and *N. gonorrhoeae* confirms the clinical observation of their concomitant isolation in about a third of cases of pelvic inflammatory disease.¹⁻⁶ Such synergy between aerobic and anaerobic organisms was observed between other bacterial species¹⁰ and may be due to protection from phagocytosis and intracellular killing,¹¹ production of essential growth factors,¹² and lowering of oxidation reduction potentials in host tissues.¹³

The ability of *Bacteroides* spp. to induce abscesses cor-

relates with the presence of capsular material. This phenomenon was observed by us in previous studies^{11, 15} and also occurs in subcutaneous abscesses induced by *N. gonorrhoeae*. The conversion of cervical nonencapsulated strains to encapsulated ones has also been observed in patients.¹⁶ Such a transition occurred in *N. gonorrhoeae* isolates recovered from the fallopian tubes or cul-de-sac and indicates the increased virulence of encapsulated strains.

The virulence of *N. gonorrhoeae* may account for the development of pelvic inflammatory disease in about a third of the women with *N. gonorrhoeae* cervical infection. This selective virulence of encapsulated *N. gonorrhoeae* present in some women may explain why some develop pelvic inflammatory disease and some do not. Because of the exceptional virulence of *N. gonorrhoeae*, it is assumed that it is responsible for initiating cervical infection. However, a significant proportion of women with *N. gonorrhoeae*-associated pelvic inflammatory disease have either no bacteria or organisms other than *N. gonorrhoeae* isolated from the fallopian tube, and the role of *N. gonorrhoeae* is not clear when it is isolated from the cervix but not from the fallopian tubes.

The decrease in the proportion of patients from whom *N. gonorrhoeae* are recovered when symptoms have been present for more than 7 days has led to the theory that *N. gonorrhoeae* tubal infection has two stages: an initial phase when the *N. gonorrhoeae* produce the infection and a later stage when the organisms are isolated infrequently. The decreased rate of recovery in the later stage coincides with our finding of the disappearance of *N. gonorrhoeae* from mixed subcutaneous abscesses and the inability of *N. gonorrhoeae* to grow intraperitoneally. Conversely, *Bacteroides* spp. induced intraperitoneal and subcutaneous abscesses, especially when they possessed a capsule.

Of particular interest was the observed ability of encapsulated *N. gonorrhoeae* to induce the conversion of slightly encapsulated *Bacteroides* spp. to heavily encapsulated ones. This phenomenon may represent the clinical chain of events that occurs after cervical *N. gonorrhoeae* infection, which may lead to tubal or pelvic inflammation. In this fashion a nonvirulent *Bacteroides* that is part of the normal vaginal flora can become virulent after exposure to *N. gonorrhoeae*; however, the *N. gonorrhoeae* that eventually did not survive were able to induce encapsulation of the *Bacteroides* spp. Similarly, the conversion of nonencapsulated *N. gonorrhoeae* after coinoculation with an encapsulated *Bacteroides* isolate may explain the increased virulence of *N. gonorrhoeae* isolates that are recovered from the tubes or cul-de-sac.¹⁶

It is postulated that a selection process was responsible for this phenomenon because a few encapsulated organisms were present among the organisms that

eventually became heavily encapsulated. The presence of heavily encapsulated bacteria was probably sufficient to prevent phagocytosis of the slightly encapsulated isolate, thus allowing it enough time to produce a protective capsule.^{14, 15}

The present study explores some of the variables in mixed infections that involve *N. gonorrhoeae*. Although the results do not seem to clearly illustrate the dynamics of the infectious process, several of the relationships between the pathogens are highlighted. Whether *N. gonorrhoeae* are essential for the infection to occur is yet to be determined. However, the synergistic relationship between *Bacteroides* and *N. gonorrhoeae* and the inability of *N. gonorrhoeae* to persist in chronic infections are in conformance with clinical findings.

The true relationship between the organisms contributing to pelvic inflammatory disease may be more complicated than the one explored in this study. *Chlamydia trachomatis* can also participate in the infectious process and contribute to its chronicity.⁸ Further work is needed to evaluate the relationship between *C. trachomatis*, *Bacteroides* spp., and *N. gonorrhoeae*.

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Modification of maternal and fetal oxygenation with the use of tracheal gas infusion

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A new technique is described for producing changes in fetal blood gases in the chronically instrumented pregnant sheep. Gas mixtures were infused directly into the maternal trachea. Maternal and fetal carotid arterial blood gases and pH were measured. Air infusion at $16 \text{ L} \cdot \text{min}^{-1}$ produced no change. Oxygen infusion caused significant increases in maternal PaO_2 at $2 \text{ L} \cdot \text{min}^{-1}$ and in fetal PaO_2 at $4 \text{ L} \cdot \text{min}^{-1}$. Nitrogen infusion significantly decreased maternal and fetal PaO_2 at $4 \text{ L} \cdot \text{min}^{-1}$. During $4 \text{ L} \cdot \text{min}^{-1}$ oxygen infusion, maternal PaO_2 rose rapidly to plateau at $314 \pm 47 \text{ mm Hg}$ at 4 minutes and fetal PaO_2 rose to plateau at $28.7 \pm 2.8 \text{ mm Hg}$ after 7 to 8 minutes. Maternal PaO_2 fell to $56.4 \pm 4.3 \text{ mm Hg}$ during nitrogen infusion ($4 \text{ L} \cdot \text{min}^{-1}$) while fetal PaO_2 fell to $15.9 \pm 1.8 \text{ mm Hg}$. Continuous infusion for 5- to 6-hour periods produced a consistent rise in maternal PaO_2 during oxygen infusion and a consistent decrease during nitrogen infusion. Tracheal infusion of gases can be used to change maternal and fetal PaO_2 rapidly and predictably. (*Am J Obstet Gynecol* 1986;155:429-35.)

Key words: Fetus, hypoxia, hyperoxia, fetal monitoring

The chronically instrumented pregnant sheep is commonly used to study fetal physiologic responses to various experimental situations because of the relative ease with which both fetus and ewe can be instrumented and monitored over long periods of time. Fetal hypoxia has been used as a challenge to investigate fetal endocrine responses, such as secretion of adrenocorticotropin,¹ catecholamines,² and arginine vasopressin.³ Fetal cardiovascular responses⁴⁻⁷ and alterations in fetal neurophysiologic functions such as fetal breathing movements⁸ have also been extensively studied.

The commonly used method of inducing hypoxia is to place a transparent bag loosely over the head of the ewe. Flow rates of different gases as high as $40 \text{ L} \cdot \text{min}^{-1}$ are flushed through the bag to alter its gas content, thereby changing the composition of air inspired by the ewe.⁸ When hypoxic gas mixtures, such as 10% oxygen, 3% air, and 87% nitrogen, are flushed through the bag, this method is effective in lowering maternal and fetal arterial partial pressures of oxygen (PaO_2). However, it is sometimes difficult to place the bag or face mask over the head of the ewe, and the ewe is always disturbed to varying degrees. Thus, when results from such experiments are interpreted, consideration must be given to the effect of maternal stress as a factor in addition to the effect of hypoxia.

Small changes in fetal PaO_2 of the order of 2 to 5 mm Hg occur spontaneously during the final third of gestation in association with myometrial contractures.⁹ To investigate the physiologic effects of contracture-related hypoxia on the fetus it is necessary to develop

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Table I. Gestational ages at the time of instrumentation and days after operation on which different infusions were performed

	Sheep No.			
	67	78	85	114
Gestational age at instrumentation (days)	124	117	117	127
Days after instrumentation for:				
Stepwise oxygen	2	2	2	4
Stepwise air	3	3	3	5
Stepwise nitrogen	2	2	2	5
Repeated oxygen	15-16	—	5-6, 7-8	12-14
Repeated air	—	—	8-9	11-12
Repeated nitrogen	—	—	6-7	9-11
Long-term continuous oxygen	8	—	15, 16	7, 8
Long-term continuous air	7, 11	—	10	—
Long-term continuous nitrogen	10	—	14	6

Table II. Significance of changes in fetal and maternal Pa_{O_2} , Pa_{CO_2} , and pH with air, oxygen, and nitrogen infusion

Infused gas	Maternal			Fetal		
	Pa_{O_2}	Pa_{CO_2}	pH	Pa_{O_2}	Pa_{CO_2}	pH
Air	NS	NS	NS	NS	NS	NS
Flow	—	—	—	—	—	—
Oxygen	$\uparrow p < 0.001$	NS	NS	$\uparrow p < 0.001$	NS	$\downarrow p < 0.05$
Flow	0-2	—	—	2-4	—	Only 8
Nitrogen	$\downarrow p < 0.001$	$\downarrow p < 0.005$	$\uparrow p < 0.001$	$\downarrow p < 0.001$	$\downarrow p < 0.001$	$\downarrow p < 0.005$
Flow	4	10	10	4	10	*

Arrow indicates direction of change. Probabilities are for two-way analysis of variance to look at trends in all the points; NS = not significant at $p = 0.05$ level. The flow rates (in liters per minute) at which differences from control occur at the $p = 0.05$ level are for two-sample t tests.

*No significant difference in individual flow.

an automatic system capable of simulating the Pa_{O_2} falls that may accompany contractures or preventing the falls associated with spontaneous contractures. Since the frequency of contractures can be as low as one every 2 hours and their effect on fetal behavior may be variable,¹⁰ the effect of large numbers of contractures during long periods of time have to be studied. Thus a system is needed that does not interfere with the eating or drinking habits of the sheep, does not need continuous supervision, and provides rapid and controllable changes in fetal Pa_{O_2} . Methods in which a bag is placed over the head of the ewe are obviously unsuitable.

This article describes a technique that permits maternal and fetal arterial blood gas composition to be changed. The experiments were designed first to test if infusion of gas into the maternal trachea was feasible in the chronic fetal sheep preparation and second to determine if such infusion would produce rapid, predictable changes in maternal and fetal oxygen tension.

Material and methods

Animal care. Four pregnant Rambouillet-Columbia ewes (body weight 49 to 58 kg) of known gestational

ages were used. At 117 to 127 days of gestation, instrumentation was carried out with the animals under general anesthesia. A sterile vinyl catheter (3.5 mm outer diameter and 2.5 mm inner diameter) was introduced into the space between the seventh and eighth tracheal rings via an aseptic midline ventral neck incision. The incision position was made more caudal if there seemed a risk of incising the balloon on the endotracheal intubation tube, which could be palpated through the intact trachea. The catheter was then passed into the trachea so that the tip was 20 cm caudal to the space between the seventh and eighth tracheal rings. The tracheal catheter was sutured to subcutaneous tissue adjacent to the incision and also to the skin after closure. The size of the incision was kept to a minimum to prevent gas leakage during infusions. The same skin incision was used to place catheters in the maternal carotid artery and jugular vein and an oxygen-sensitive Clarke electrode (Searle, High Wycombe, UK, 5F gauge) in the maternal carotid artery. With the use of techniques previously described,¹¹ sampling catheters were placed in the fetal carotid and jugular vessels. A Clarke electrode⁹ was also placed in the fetal carotid

for PO_2 measurement. A thermistor was sutured to the uterus to investigate the effect of infusion of non-warmed gas. Details of experimental animals are given in Table I. At the end of the experiments, the sheep were killed with an overdose of T-61 (Hoechst, Somerville, New Jersey) and necropsy was performed.

Gases were not warmed, humidified, or filtered and were passed through a standard two-stage regulator with an output pressure of 17 kPa (25 pounds per square inch) and a standard ball valve flowmeter. Where the flow of gas was controlled automatically, an electrically operated solenoid valve (Type B2LX49, Skinner Electric Valves, New Britain, Connecticut) was interposed between the regulator and the flowmeter. The solenoid was placed on the gas cylinder to minimize the length of tubing pressurized to the regulator outflow pressure and reduce gas surges when the valve opened. While not in use the tracheal catheter was capped to minimize accumulation of fluid.

Modification of fetal and maternal blood gases.

Three different protocols for gas infusion were adopted. Initially, gases were altered in a stepwise manner to obtain a dose-response relationship between flow rate and the changes in fetal and maternal blood gas levels. In a second series of experiments, repeated infusions at one flow rate were automatically switched on and off to derive timing relationships between gas flow and maternal and fetal PaO_2 changes. These repeat infusions were performed in order to examine small changes in certain maternal and fetal variables with the use of signal averaging techniques. Such small changes would normally be masked by variability from non-infusion-related sources. Last, long-term infusions were performed to see if maternal and fetal PaO_2 levels could be maintained and if the ewes remained unperturbed during several hours of infusion.

Stepwise dose-response infusions. Air or oxygen was infused into the tracheal catheter for 10 minutes at the following rates: 0, 0, 2, 4, 6, 8, 10, 12, 14, 16, 10, 4, and 0 $\text{L}\cdot\text{min}^{-1}$; or nitrogen was infused at 0, 0, 2, 4, 6, 8, 10, 8, 6, 4, 2, and 0 $\text{L}\cdot\text{min}^{-1}$; higher nitrogen rates were avoided in case they resulted in permanent hypoxic damage to the fetus. Eight minutes into each period maternal and fetal arterial blood samples were collected for gas tension and pH determination with a Radiometer ABL2. At least 3 hours was allowed before infusion of a different gas was started. Each ewe was tested once with each gas. Mean and standard deviation of maternal and fetal carotid PaO_2 , PaCO_2 , and pH were calculated at each gas flow rate, including the three control periods.

Repeated infusions. In three pregnant sheep, infusions of nitrogen, air, or oxygen at a flow rate of 4 $\text{L}\cdot\text{min}^{-1}$ were repeated for either 8.5 or 15 minutes every hour.

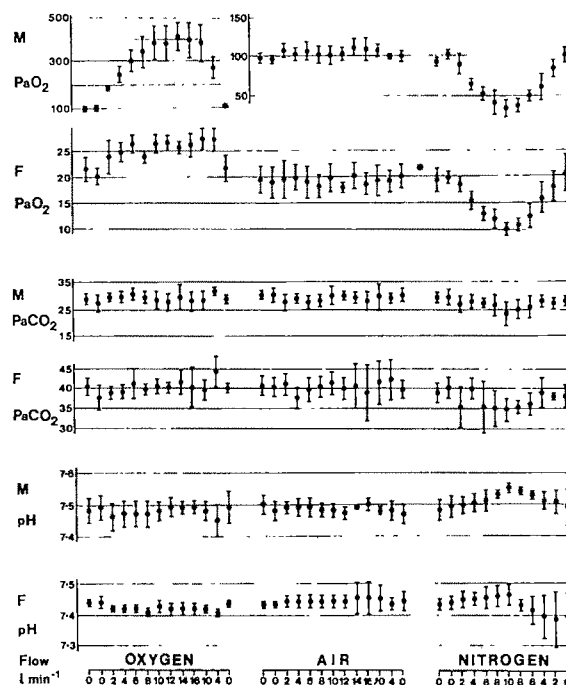


Fig. 1. Average maternal (M) and fetal (F) PaO_2 in millimeters of mercury, PaCO_2 in millimeters of mercury, and pH values $\pm$ SD from four sheep infused with oxygen (left), air (middle), or nitrogen (right) at the flow rates indicated. Samples were taken after 8 minutes at each flow rate.

The experiments were repeated in one ewe after delivery with only maternal samples used. There were 234, 187, and 59 hours of experiment on nitrogen, oxygen, and air, respectively. All variables were sampled at 32 Hz, averaged for 8-second intervals, and stored in data files 1 hour long. Signal averaging and other analysis, including Clarke electrode calibrations, were performed on a VAX11/750. Signal averages were calculated by averaging the same data point from each file for each channel to give the mean and standard deviation activity of the channel for each 8-second interval throughout the 1-hour infusion and recovery period.

Long-term tracheal infusions. Nitrogen or oxygen was infused in three ewes and air in two for 5 to 7 hours at 4 or 8 $\text{L}\cdot\text{min}^{-1}$. Nitrogen at 8 $\text{L}\cdot\text{min}^{-1}$ was infused last in case the fetus died during the procedure. Biophysical data were collected as with the repeated infusions, and maternal and fetal blood samples were taken for blood gas analysis before the infusion and 40 minutes after the start, just before the end, and 40 minutes after the end of the infusion. The animals were observed for considerable lengths of time during these infusions for any changes in their behavior.

Statistical analysis and data presentation. Data throughout are presented as mean $\pm$ SD. Significant differences between individual points for blood gas

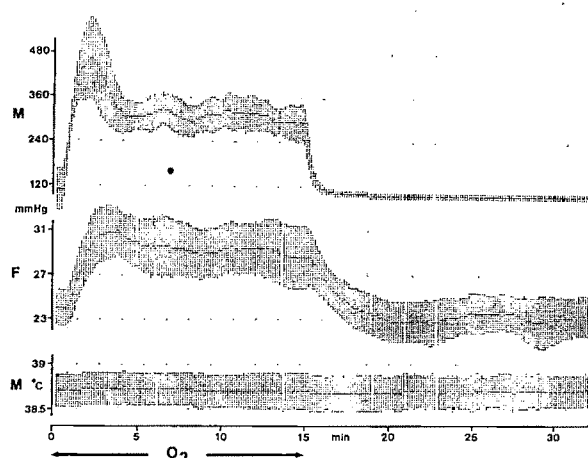


Fig. 2. Signal average of maternal (M) carotid PaO_2 (top trace), fetal (F) carotid PaO_2 (middle trace), and maternal temperature (lower trace) during 26 15-minute 100% oxygen infusions at 4 $\text{L}\cdot\text{min}^{-1}$ spaced at hourly intervals in one ewe at 139 days' gestation and 12 days after operation.

changes at different flow rates were calculated with the two-sample t test. Trends in individual variables were examined by use of two-way analysis of variance.

Results

Stepwise infusion. Fig. 1 shows the pooled data from four pregnant ewes in which either oxygen, air, or nitrogen was infused for 10 minutes at the indicated flow rates. Table II shows which infusion procedures affected maternal and fetal PaO_2 , PaCO_2 , and pH. Maternal oxygen infusion raised both fetal and maternal PaO_2 and it is doubtful that it altered fetal pH despite one statistically significant value at 8 $\text{L}\cdot\text{min}^{-1}$. Maternal and fetal PaO_2 levels rose from 101 ± 8 and 20 ± 1 to 395 ± 66 and 26 ± 2 mm Hg, respectively, at the 16 $\text{L}\cdot\text{min}^{-1}$ infusion rate.

Nitrogen infusion, however, resulted in hypoxia in both fetus and mother and, since no attempt was made to counteract hypocapnia by infusing some carbon dioxide, both maternal and fetal PaCO_2 fell, while pH increased. Mother and fetus became significantly hypoxic at between 2 and 4 $\text{L}\cdot\text{min}^{-1}$ of infused nitrogen. At 10 $\text{L}\cdot\text{min}^{-1}$, the PaO_2 values fell from 102 ± 4 and 20 ± 1 to 36 ± 9 and 10 ± 1 mm Hg, respectively. Maternal and fetal PaCO_2 was significantly lower than control only at the maximum flow rate of 10 $\text{L}\cdot\text{min}^{-1}$, where values fell from 30 ± 2 and 40 ± 2 to 24 ± 4 and 35 ± 3 mm Hg, respectively.

Repeated infusions. Since a flow of 4 $\text{L}\cdot\text{min}^{-1}$ of nitrogen reduced fetal PaO_2 from 20.1 ± 1.1 to 15.7 ± 1.5 mm Hg in the four animals (Fig. 1), we performed repeated infusions of test gases at this flow rate in the same animals (see Table I) to assess the variability of the response and examine timing relationships between

changes in gas flow and maternal and fetal variables. Although the repeated infusions documented in Table I were performed for 8 minutes, one animal was infused for 15 minutes every hour since fetal PaO_2 had not equilibrated during the 8-minute nitrogen infusion. The 15-minute infusion is illustrated in Figs. 2, 3, and 4 for oxygen, nitrogen, and air. The gas changes in the first 8 minutes are identical to those in the 8-minute infusions. The asymptotic nature of the changes in both fetal and maternal PaO_2 falls during hypoxia is clearly shown. All timing accuracies must be interpreted with a sample interval of 8 seconds in mind.

Oxygen. Fig. 2 is a signal average of maternal and fetal PaO_2 measured with indwelling PO_2 electrodes and maternal temperature during 26 hour-long experiments where oxygen was infused for 15 minutes every hour at 4 $\text{L}\cdot\text{min}^{-1}$. There was a rapid rise in maternal PaO_2 from 97 ± 4 mm Hg starting 24 to 32 seconds after the gas was turned on to a peak at 460 ± 106 mm Hg after 129 seconds. The PaO_2 then fell to a plateau at 314 ± 47 mm Hg after about 240 seconds in a damped oscillation with a period of about 256 seconds. When oxygen administration was stopped at the end of the 15-minute infusion, maternal PaO_2 began to fall within 8 seconds, reaching 50% of the plateau in 25 seconds and 5% in about 120 seconds, a considerably quicker response than when the gas was turned on.

Fetal PaO_2 started to rise 48 seconds after the oxygen infusion started. It rose from 23.7 ± 1.5 mm Hg to a peak after 224 seconds of 30.6 ± 1.9 mm Hg. It then fell to a plateau of 28.7 ± 2.8 mm Hg about 7 to 8 minutes after the start of the infusion. When the gas flow was stopped, the fetal PaO_2 began to fall rapidly in about 32 to 40 seconds. It had fallen 50% of the way from its plateau value in 80 to 100 seconds. These figures can only be approximate since there was an underlying damped oscillation after changes in gas flow with a period of about 7 minutes (see Fig. 2). There was also a depression of fetal PaO_2 after hyperoxia, which took some 20 minutes to recover. The high standard deviation in fetal PaO_2 , which was present even long after the infusion was over, reflects the natural variation in fetal PaO_2 associated with non-infusion-related activities (for instance, myometrial activity, fetal movement, breathing, and sleep-state changes).

Nitrogen. Data from 48 hour-long experiments where nitrogen was infused at 4 $\text{L}\cdot\text{min}^{-1}$ for 15 minutes every hour were processed by signal averaging techniques (Fig. 3). Maternal PaO_2 fell from 94 ± 4.3 to 56 ± 4.3 mm Hg in an exponential decay after a 16- to 24-second delay. Fifty percent of the fall was complete in 42 seconds. Fetal PaO_2 fell from 24.2 ± 1.6 to 15.9 ± 1.8 mm Hg. This fall started 48 seconds after the gas was turned on. When the nitrogen flow ceased, both maternal and fetal PaO_2 rose more rapidly but in the same pattern as

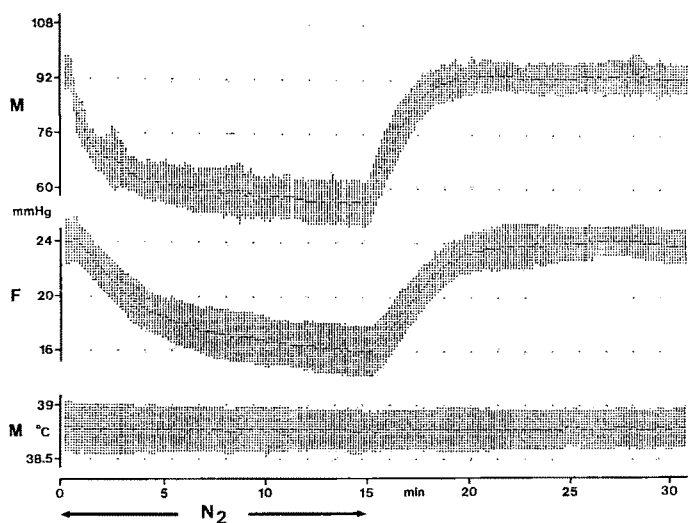


Fig. 3. Signal average of maternal (*M*) carotid PaO_2 (*top trace*), fetal (*F*) carotid PaO_2 (*middle trace*), and maternal temperature (*lower trace*) during 48 15-minute nitrogen infusions at $4 \text{ L} \cdot \text{min}^{-1}$. The gas infusions were spaced at hourly intervals in one ewe at 136 days' gestation and 9 days after operation.

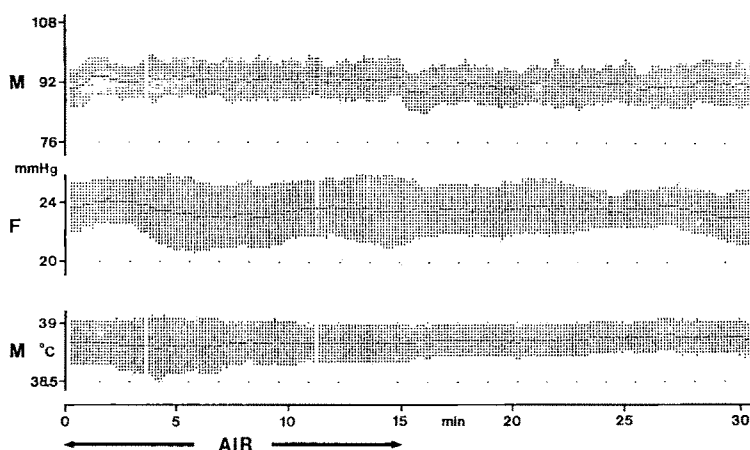


Fig. 4. Signal average of maternal (*M*) carotid PaO_2 (*top trace*), fetal (*F*) carotid PaO_2 (*middle trace*), and maternal temperature (*lower trace*) during 29 15-minute air infusions at $4 \text{ L} \cdot \text{min}^{-1}$ spaced at hourly intervals in one ewe at 138 days' gestation and 11 days after operation.

the falls. Fifty percent recovery was achieved in 80 and 114 seconds for ewe and fetus, respectively, and 95% recovery took 3 to 4 and 5 to 6 minutes, respectively.

Air. Fig. 4 shows a signal average from 29 experiments in which air was infused for 15 minutes at $4 \text{ L} \cdot \text{min}^{-1}$. There was a slight but consistent rise in maternal PaO_2 lasting the duration of the infusion. Maternal PaO_2 rose from 91 ± 5 to $93.5 \pm 5 \text{ mm Hg}$. Fetal PaO_2 did not change from resting levels of $23.7 \pm 1.5 \text{ mm Hg}$.

Temperature. There was no change in maternal temperature in any of the animals infused with any gas. Changes associated with maternal posture were apparent, but since the posture of the ewe and the presence

of infusion seem unrelated, these average out to a constant $38.77 \pm 0.22^\circ \text{C}$. None of the animals were observed to shiver during repeated infusions.

Long-term tracheal infusion. Ewes ate, drank, eructated, and ruminated during infusions. There was no fall in maternal or fetal temperature. Data from the sheep in which these infusions were carried out are given in the lower part of Table I. Two nitrogen flow rates were used, 4 and $8 \text{ L} \cdot \text{min}^{-1}$. Maternal PaO_2 was reduced from 97 ± 4 and 103 ± 1 to 57 ± 7 and $51 \pm 21 \text{ mm Hg}$, respectively, 40 minutes after the start of the infusion for the two rates. After 5 to 6 hours of infusion, maternal PaO_2 values were 62 ± 8 and $42 \pm 4 \text{ mm Hg}$ when the gas was turned off and rose to

Table III. Fetal PaO₂, PaCO₂, and pH from samples before, 1 hour into, at the end of, and 1 hour after maternal tracheal infusion of either nitrogen or oxygen at 4 or 8 L · min⁻¹

Samples	Value	Before infusion	1 hr after start	Before end	1 hr after end
Nitrogen, • 4 L · min ⁻¹ (n = 3)	PaO ₂	22.4 ± 2.3	17.6 ± 0.7	17.9 ± 1.6	22.9 ± 0.7
	PaCO ₂	45.5 ± 7.3	41.2 ± 1.9	37.9 ± 3.5	41.4 ± 5.4
	pH	7.35 ± 0.03	7.35 ± 0.05	7.38 ± 0.03	7.37 ± 0.03
Nitrogen, 8 L · min ⁻¹ (n = 2)	PaO ₂	19.7, 20.5	10.7, 14.2	11.9, 12.4	20.9, 16.6
	PaCO ₂	41.0, 43.4	35.5, 36.6	33.7, 37.2	34.6, 46.1
	pH	7.36, 7.40	7.37, 7.35	7.27, 7.23	7.31, 7.41
Oxygen, 4 L · min ⁻¹ (n = 3)	PaO ₂	22.9 ± 2.6	24.2 ± 1.4	27.6 ± 1.7	21.5 ± 2.8
	PaCO ₂	43.1 ± 1.0	49.5 ± 3.2	47.6 ± 5.5	42.1 ± 3.9
	pH	7.35 ± 0.04	7.32 ± 0.04	7.32 ± 0.05	7.35 ± 0.05
Oxygen, 8 L · min ⁻¹ (n = 3)	PaO ₂	21.8 ± 1.3	25.1 ± 0.1	27.6 ± 2.2	22.8 ± 2.3
	PaCO ₂	42.9 ± 3.3	45.3 ± 4.8	46.8 ± 1.5	41.8 ± 2.7
	pH	7.34 ± 0.03	7.34 ± 0.04	7.31 ± 0.03	7.37 ± 0.03

Values are mean ± SD except in 8 L · min⁻¹ nitrogen panel where individual values are given for two animals. Each infusion lasted from 5 to 6 hours.

109 ± 14 and 110 ± 12 mm Hg 40 minutes later. Oxygen infused to the mother under the same protocol increased maternal PaO₂ from 96 ± 12 and 108 ± 12 to 240 ± 18 and 319 ± 43 mm Hg. When oxygen infusion was stopped, PaO₂ values fell from 220 ± 6 and 378 ± 51 to 99 ± 14 and 102 ± 7 mm Hg, respectively, for the two flow rates. Fetal blood gas values are shown in Table III.

Findings at necropsy. Necropsy revealed an area of inflammation 1 to 2 cm in diameter at the site of entry of each infusion catheter through the tracheal mucous membrane. No other abnormalities were found in the respiratory tract except for two small (1 to 2 cm in diameter), chronic, encapsulated, inspissated abscesses in the lung parenchyma of each of two sheep (No. 67 and No. 85). This finding is not unusual in our sheep and seems unrelated to the tracheal catheter or infusion. Sheep No. 114 delivered a live fetus at 142 days' gestation and was not pregnant at the time of necropsy.

Incidence of coughing. Ewes undergoing infusion occasionally coughed when the gas was first infused after operation or after several days without infusion. This minor irritation seemed to be related to forcing accumulated fluid from the tracheal catheter into the trachea. Sheep undergoing switched repeated infusions rarely coughed and seemed unaware that the infusion had started even if eating or drinking at the time.

Comment

The techniques described permit either increase or decrease of maternal and fetal oxygenation with the use of tracheal gas infusion in the chronic fetal sheep preparation. Neither air nor oxygen infusion changed PaCO₂. The PaCO₂ decrease associated with nitrogen treatment was probably related to increased ventilation after hypoxia. The hypocapnia might be corrected with carbon dioxide mixed with the nitrogen.

Repeated oxygen infusions produced a damped oscillation in maternal PaO₂ with decay to a plateau, which was maintained for at least 6 hours. This was probably in response to a reflex decrease in alveolar ventilation at high maternal PaO₂. The reduced peak seen in the fetal PaO₂ may result from the lag between fetal and maternal PaO₂, since by the time the fetal PaO₂ is about to peak, the maternal PaO₂ has already started to fall.

Variation in response to a given flow between sheep (Fig. 1) may be due to differences in size. It is necessary to adjust the gas flow rate to obtain the specific change in blood gas tensions desired.

The speed with which fetal and maternal oxygen tensions respond to alterations in inspired gas makes possible clamping of fetal PaO₂ levels or simulation of PaO₂ falls associated with myometrial contractures.⁹ The rapid response also makes it simpler to achieve a desired level of hypoxia than with a bag over the head. Subjecting the fetus to deep hypoxia is also safer since removal of hypoxic gas or replacement with oxygen can be done almost instantly. The bag method is slower and more expensive, requiring high flow rates of up to 40 L · min⁻¹.^{1,2,8} A major advantage of the tracheal catheter is that hypoglycemia and thirst are probably avoided since eating and drinking behavior seems unaffected. This method is much easier to implement than enclosing the sheep, with its feed and water, in a gas-tight container.

We believe that this rapidly responding preparation, combined with continuous PO₂ measurement with the use of PO₂ electrodes,⁹ will be useful in evaluating the cause and effects of small changes in fetal oxygenation that previously were either ignored or could not be recorded.

We are grateful to Karen Moore for her help with the manuscript.

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Nonstress and contraction stress testing of baboon fetuses

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This study of six pregnant baboons at 133 to 175 days of gestation indicates that all of 26 nonstress tests (two to seven per animal) and all of eight contraction stress tests (one or two per animal) were successfully performed despite the need for sedation of the dam with ketamine. (*AM J OBSTET GYNECOL* 1986;155:435-6.)

Key words: Baboon, nonstress test, contraction stress test, pregnancy

Obstetric procedures designed to evaluate the fetus have become a part of obstetric practice in both human and nonhuman primates. Ultrasonography and amniocentesis have been shown to be of value. On the other hand, evaluation of fetal well-being by nonstress (NST) and contraction stress test (CST) would appear less promising in large nonhuman primates wherein cooperation may be obtained only at the expense of sedation. We performed this investigation to determine whether NST and CST were feasible in baboons sedated with ketamine.

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Material and methods

Six pregnant baboons (*Papio cynocephalus* or *P. Hamadryas*) were initially sedated with 10 mg/kg of ketamine administered intramuscularly. This dosage was repeated as required to maintain the animal calm and comfortable. Uterine contractions were monitored externally with a strain gauge and fetal heart rate was monitored with a sonographic transducer; both signals were recorded simultaneously on a Hewlett-Packard cardiotocograph. An NST was judged successful and reactive if stimulation of the fetus produced an increase in fetal heart rate of at least 15 bpm over the baseline value, lasting a minimum of 15 seconds.¹ A CST was judged successful if an intravenous infusion of oxytocin produced at least three uterine contractions within 10 minutes.²

Results

A total of 26 NSTs (two to seven per animal, at weekly intervals) were attempted at 133 to 175 days of gestation

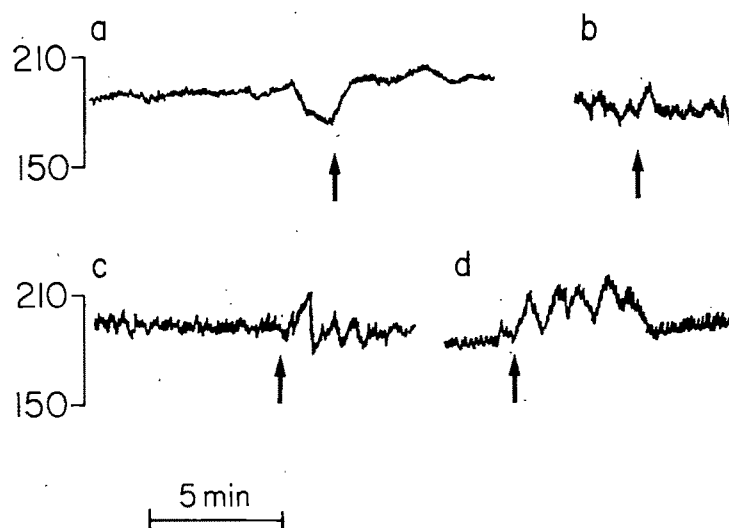


Fig. 1. Reactive NSTs elicited by spontaneous fetal movements (*a*), by pressure on the uterus (*b*), by pressure on the fetal head (*c*), and by amniocentesis (*d*). Each triggering event is marked by a vertical arrow. Tracing shows fetal heart rate only.

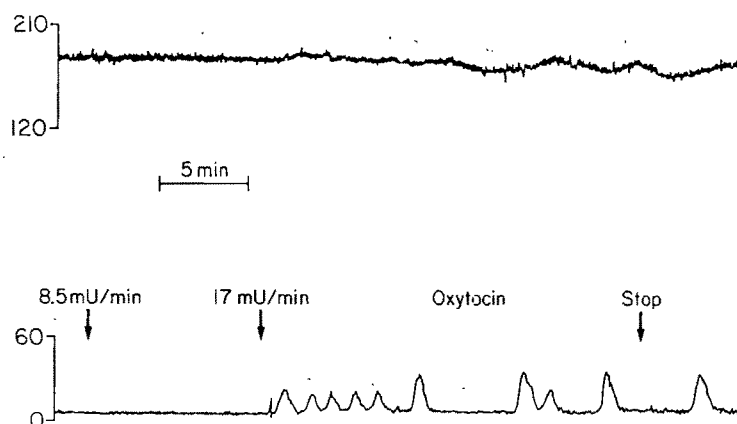


Fig. 2. CST elicited by oxytocin (17 mU/min). Tracing shows fetal heart rate (*top*) and strength of uterine contractions (*bottom*).

(term 180 days). All were successful. Stimulation of the fetal heart rate could be obtained by spontaneous fetal movements, by transabdominal pressure on the uterus, by transabdominal pressure on the fetal head, or by amniocentesis (Fig. 1). A total of eight CSTs (one or two per animal) were attempted at 147 or 175 days of gestation. All were successful. Adequate uterine contractions occurred with oxytocin doses ranging from 1.7 to 34 mU/min, but a dose of 17 mU/min was most commonly effective (Fig. 2).

Comment

These data suggest three conclusions. First, although ketamine does cross the placenta, its effect on the fetus is not known. Fetal movements and breathing persist as seen by real-time ultrasonography. Chemical dilution and clearance studies indicate that fetal ingestion of am-

niotic fluid also is not impaired. Successful NSTs and CSTs indicate that heart rate reactivity is also maintained. Second, the uterus of the baboon appears to respond to similar doses of oxytocin in a manner similar to that of the human uterus. Third, it is feasible to perform NSTs and CSTs in nonhuman primates despite the need for sedation. Whether the results of these tests have the same meaning as in human pregnancies remains to be determined. In view of the considerable similarities between fetoplacental-uterine units in baboon and human, one might venture an optimistic opinion.

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Arterial PO₂, PCO₂, and pH versus transcutaneous PO₂ and PCO₂ and tissue pH in the fetal dog

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In the fetal dog, simultaneous recording by transcutaneous PO₂ and PCO₂ and tissue pH electrodes were compared to corresponding arterial values during hypoxic episodes produced by occlusion of the maternal abdominal aorta. Before occlusion, the differences between the paired values were minimal. Under anoxic conditions, expected changes in the peripheral circulation were observed. However, the transcutaneous PO₂ was lower, the transcutaneous PCO₂ much higher, and the tissue pH much lower than in blood. Continuous electrodes demonstrate changes resulting from gas and hydrogen ion coming from cells more readily than blood because they are closer to the former. Additionally, carbon dioxide and hydrogen ion are buffered to a greater degree in blood than in cells. Consequently, under conditions of stress and active metabolism, PCO₂ is higher while PO₂ and pH are lower in cells than in blood. When compared with monitoring of gases and acid-base balance through blood sampling, continuous transcutaneous and intracutaneous monitoring would seem to be more representative of the environment at the cellular level. (AM J OBSTET GYNECOL 1986;155:437-43.)

Key words: tcPO₂, tcPCO₂, tpH, fetal dog

Intermittent fetal scalp blood sampling for pH determination is an invasive procedure, at times difficult to perform and associated with occasional complications.¹ An alternative method for acid-base monitoring, which is currently used in neonates, is transcutaneous monitoring of PO₂ and PCO₂ along with continuous tissue pH, for which there is limited experience in humans. If these techniques could be perfected and demonstrated to be accurate, a breakthrough in fetal surveillance similar to the one achieved with continuous fetal heart rate monitoring² would be within the realm of possibility. Continuous monitoring of these gas and acid-base values, however, still presents numerous technologic difficulties.¹ Furthermore, data derived in this manner seem to be different from the corresponding data determined through blood analysis at the same time, making their interpretation difficult. For these reasons, continuous monitoring of tissue pH (tpH) and of transcutaneous PO₂ and PCO₂ (tcPO₂ and tcPCO₂) is still under investigation.^{3,4}

The purpose of the present study is to compare these two techniques in the fetal dog under normal and hypoxic conditions. Similar investigations have already

been reported^{3,5} but are limited in scope in that they consider only one of these three elements, either tpH, or tcPO₂, or tcPCO₂. In this study continuous data from all three are simultaneously recorded from the same fetus.

Material and methods

Eleven pregnant bitches with gestational ages varying from 7 to 9 weeks were investigated in this study. Data from the first three animals were not used in the computation of results since they served primarily to perform certain modalities that will be described later. The other eight dogs were prepared identically according to an animal preparation that has been described previously.³ Briefly, anesthesia was induced with 100 to 200 mg of pentobarbital sodium injected intravenously, and an endotracheal tube was placed and connected to a Harvard respirator delivering, at a rate of 12 respirations per minute, 300 to 400 ml of either room air or oxygen at different concentrations. Continuous anesthesia was maintained with succinylcholine by intravenous drip (500 mg in 500 ml of normal saline solution) and repeated intravenous injection of ketamine hydrochloride, 1 mg/kg of body weight. A cuff of a blood pressure machine (Kontron 1022, Everett, Massachusetts) was placed around an anterior limb allowing intermittent recording of blood pressure. A left paralumbar incision exposed the abdominal aorta extraperitoneally and an 8 to 10 mm flow probe (Statham SP2202) and a 10 mm vascular occluder (Rhodes, Woodland Hills, California) were placed around it, thus

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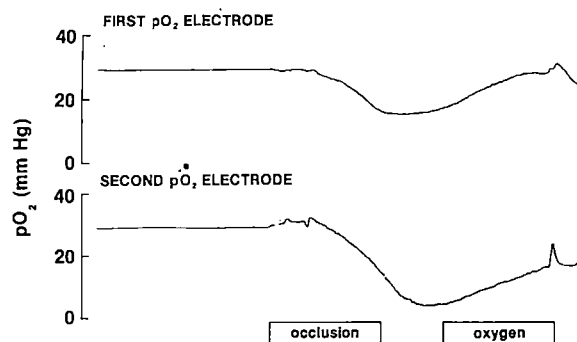


Fig. 1. Two PO_2 electrodes were implanted in the same fetus. tcPO_2 went down during aortic occlusion, then up during oxygenation. The two variation curves were almost identical, thus confirming the validity of the recordings.

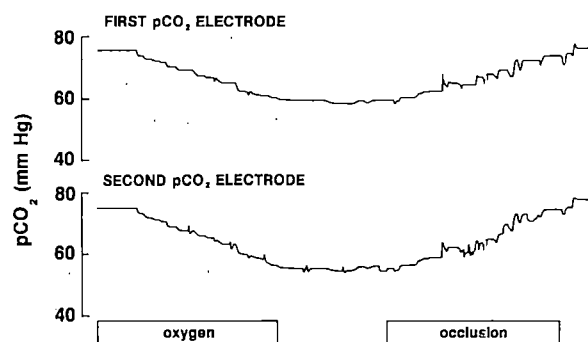


Fig. 2. Two PCO_2 electrodes were implanted in a previously asphyxiated fetus. tcPCO_2 went down during oxygenation, then up during aortic occlusion. The two variation curves were almost identical, again confirming the validity of the recordings.

allowing different degrees of aortic occlusion while continuously monitoring aortic blood flow. The paralumbar incision was closed, and the animal was turned to the supine position. Laparotomy was performed, and a uterine horn was incised over the lower back of one of the fetuses. One fetal hind limb was exteriorized, and a polyvinyl catheter was inserted into the femoral artery and advanced into the descending aorta; the hind limb was replaced in the uterine horn, with the femoral catheter delivered outside. The incised uterine edges were sutured in a circular manner to the back of the fetus, exposing a circle of fetal skin about 5 cm in diameter. The loss of amniotic fluid was minimal. The tcPO_2 , tcPCO_2 , and tpH electrodes (Kontron) were calibrated with media of known concentrations.³⁻⁵ The transcutaneous electrodes were mounted on grooved plastic rings⁴ and, with the use of cyanoacrylate glue, were attached to the fetal skin that had been treated with a depilatory lotion. The transcutaneous electrodes were heated to 44° C. The tpH electrodes were also implanted according to previously described protocols.³⁻⁵ The catheter coming from the fetal femoral artery was connected through a three-way stopcock to (1) a 0.9% sodium chloride solution containing 1 unit of heparin per milliliter with the solution delivered at the speed of 1 ml/hr, (2) a Statham pressure transducer (another catheter in the amniotic cavity was used as a zero reference), and (3) a tuberculin syringe through which 0.08 to 0.1 ml of fetal blood was drawn each time.

The experimental procedure was started after the tpH , tcPO_2 , and tcPCO_2 digital displays remained stable for at least 15 minutes. Two dogs were used as controls and had only a sham aortic occlusion, but in six others the maternal aorta was rapidly and completely occluded for 4 minutes, and then the occlusion was released. Femoral arterial blood was drawn five times: initially,

just before occlusion; second, in the middle of the occlusion; third, at the termination of occlusion; and fourth and fifth, at 2 and 4 minutes after occlusion; therefore, the five blood samples were drawn at 2-minute intervals. A recovery period of 1 hour was allowed before the procedure was repeated. The corresponding values of tpH , tcPO_2 , and tcPCO_2 at the beginning of each blood sampling were carefully noted. The entire protocol was repeated in another fetus until two to three fetuses of the same litter had been investigated. In all, 15 fetuses from six litters were tested, with a total of 24 aortic occlusions and 126 blood specimens drawn for pH , PO_2 , and PCO_2 determinations. If the continuous values had not returned to normal after 1 hour of recovery, the animal was increasingly oxygenated to allow faster recovery. Individual study periods did not last more than 90 minutes for each fetus or 6 hours for each litter.

Meticulous attention was paid to maternal condition during the procedure with frequent observations of pulse and blood pressure, with appropriate hydration, and with assurance that the mother was not over-anesthetized. Blood was drawn from each pregnant dog before, during, and at the end of the study for measurement of PO_2 , PCO_2 , and pH to determine proper ventilation and hydration of the animal.

When the study of the second or third fetus (depending on whether two or three fetuses were investigated in a litter) was completed, the fetus was delivered from the uterine horn, the umbilical cord was clamped, and it was exposed to room air breathing while the three continuous electrodes were still implanted on the skin. At 5 minutes of life, the same comparative study between the fetal blood and the display from the continuous electrodes was repeated.

The blood gas and pH were determined on an automatic blood gas analyzer (Instrument Laboratory

Table I. Continuous and arterial PO₂, PCO₂, and pH associated with 4-minute aortic occlusion

	<i>Before occlusion</i>	<i>2 min occlusion</i>	<i>End of occlusion</i>	<i>2 min after occlusion</i>	<i>4 min after occlusion</i>	<i>60 min after occlusion</i>	<i>Oxygen to mother</i>	<i>10 min after birth</i>
No. of specimens	24	14	18	17	17	23	8	5
PO ₂ (mm Hg)								
Arterial	22.8 ± 0.5	18.1 ± 0.9	15.3 ± 0.6	11.0 ± 0.5	13.1 ± 0.5	22.7 ± 0.4	27.2 ± 0.9	58.6 ± 3.7
Transcutaneous	21.7 ± 0.6	16 ± 0.8	11.2 ± 0.6	6.6 ± 0.5	8.1 ± 0.6	21.2 ± 0.5	26.4 ± 1.0	52.4 ± 2.6
PCO ₂ (mm Hg)								
Arterial	41.8 ± 0.8	45.4 ± 0.9	51.9 ± 0.9	54.8 ± 0.9	52.2 ± 0.8	41.7 ± 0.8	38.3 ± 0.8	50 ± 1.5
Transcutaneous	49 ± 0.8	57.5 ± 1.1	60 ± 1.1	77.1 ± 1.2	61.2 ± 1.5	50.1 ± 0.8	45.8 ± 1.1	54 ± 1.9
pH								
Arterial	7.36 ± 0.7	7.34 ± 0.9	7.23 ± 0.8	7.18 ± 0.5	7.21 ± 0.8	7.33 ± 0.8	7.36 ± 1.7	7.35 ± 2.1
Tissue	7.34 ± 0.9	7.28 ± 0.9	7.15 ± 0.8	7.06 ± 0.8	7.13 ± 1.0	7.30 ± 0.7	7.33 ± 2.0	7.30 ± 3.4

Values are mean ± SEM.

1302) with the use of 0.09 ml of blood for a combined determination of pH, PO₂, and PCO₂.

At the end of each experimental study, the animals were put to death with intravenous pentobarbital sodium, and all the electrodes were removed and recalibrated to check for any drift.

The original three dogs were used to investigate different degrees of maternal aortic occlusion, and from these three dogs it was determined that 100% aortic occlusion for 4 minutes was ideal because it produced an acute and severe fetal anoxia; it also allowed enough time to draw fetal blood at regular intervals. These three dogs were also used to determine if the values displayed by the electrodes represented true oxygen and true carbon dioxide tension, or if they contained artifacts. This was done in the following manner: Two tcPO₂ electrodes were implanted in proximity on the same fetal skin, and hypoxia then hyperoxygenation were produced as described previously. It was assumed that if there was artifact, it most likely would not be exhibited identically by the two electrodes during a prolonged period of observation. This procedure was repeated with two tcPCO₂ electrodes. An identical technique previously reported⁵ was used to demonstrate the validity of the value displayed by the tpH electrodes.

Results

In the three original dogs, the implantation of two tcPO₂ electrodes in the same fetus showed virtually identical variation regardless of the stage of anoxia or hyperoxygenation, and the differences were minimal (Fig. 1). Similar results with two tcPCO₂ electrodes confirmed the validity of the recordings (Fig. 2).

In the two dogs that had a sham aortic occlusion,

only minimal variations in PO₂, PCO₂, and pH (both in blood and in continuous recordings) were noted during several hours of fetal observation.

Maternal condition remained stable throughout the procedure as demonstrated by the stability of the maternal heart rate, blood pressure, and blood gas and acid-base determinations.

The fetal condition also remained stable throughout the procedure. The mean fetal blood pressure, recorded between occlusions, varied between 37 and 42 mm Hg, depending on the fetus, but presented minimal variations within each fetus. The fetal heart rate, observed between occlusions by the intrafemoral pressure transducers, remained stable. Hemoglobin concentration, measured at the beginning and the end of the study, demonstrated minimal variation.

Table I summarizes the mean values of intermittent and continuous PO₂, PCO₂, and pH in relation to different stages of occlusion. Figs. 3, 4, and 5 show the paired values to be closest before occlusion of the maternal aorta. The difference progressively increases until 2 minutes after occlusion, following which recovery starts and there is a return to original values 1 hour later. Even before occlusion, the blood PO₂ is slightly higher, the blood carbon dioxide is lower and the blood pH is higher than the corresponding continuous values. Regression equations are difficult to construct because they are not linear and because the correlation coefficient seemed to vary depending on the original value of PO₂, PCO₂, and pH; a correlation coefficient would be higher with low than with high original values because the differences between the paired values are greater with low values of PO₂, PCO₂, and pH and lower with normal values of PO₂, PCO₂, and pH.

The calibration of the continuous electrodes at the

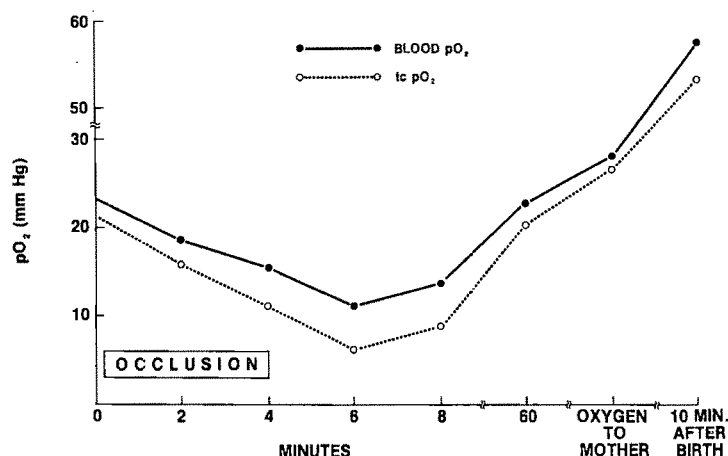


Fig. 3. Four-minute maternal aortic occlusion. Before and late after occlusion, tcPO₂ was only slightly lower than blood PO₂. During and immediately after occlusion, tcPO₂ was noticeably lower.

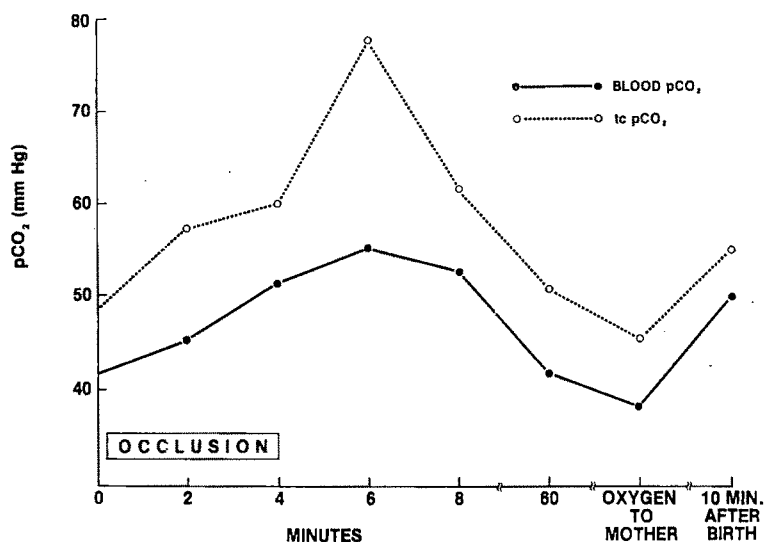


Fig. 4. Four-minute maternal aortic occlusion. Before and late after occlusion, tcPCO₂ was higher than blood PCO₂. During and immediately after occlusion, tc PCO₂ was much higher. Under stress the difference in carbon dioxide concentration between cells and blood was much greater, especially in view of the greater buffering capacity of blood (see Fig. 6).

end of the study showed a drift not exceeding ± 0.01 for tpH and ± 2 mm Hg for tcPO₂ and tcPCO₂.

Comment

The data presented in this study demonstrate that values derived from continuous monitoring are different from intermittent values in blood, with tcPCO₂ being higher and tcPO₂ and tpH being lower than the corresponding blood values. The differences between them (Δ PO₂, Δ PCO₂, and Δ pH) are greater during hypoxia than in normal conditions, and these differences are more marked for PCO₂ than for PO₂.

The electrodes seemed to be correctly implanted and they were not registering artifacts. The criteria for de-

termining correct implantation of these continuous electrodes are that, after calibration has been carried out and after the electrodes have been in place for at least 1 hour, they should repeatedly indicate the expected normal values, that these values should change appropriately during induced hypoxia or hyperoxygenation, that the values should return to baseline after an adequate period of recuperation, and that drift at the end of the experiment should be minimal. The electrodes used in this study fulfilled not only these criteria but an additional criterion: Two identical electrodes (two tpH electrodes,⁵ then two tcPO₂ electrodes, then two tcPCO₂ electrodes) were implanted simultaneously in the same fetus during hypoxia and during

hyperoxygenation; the results given by the two identical electrodes were very similar, thus confirming the validity of the values displayed by these electrodes.

Given the accuracy of transcutaneous gas and intracutaneous pH monitoring, the question is why they are so different from the blood values. Several authors have proposed multiple regression equations with different correlation coefficients and intercepts⁴⁻⁶ in an attempt to convert transcutaneous or intracutaneous values to blood values, so that the latter could be determined by measuring the former. The shortcoming of these mathematical formulas is the assumption that these values represent identical physiologic systems, which they do not.

This inability to determine what would be the blood value by measuring the continuous values, compounded with some technical difficulties in implanting these continuous electrodes, has discouraged their clinical use in obstetrics and has limited their use during pregnancy to laboratory animals.

Some authors have criticized this approach. Huch et al.³ stated that the $tcPO_2$ electrode does not measure the arterial PO_2 . Tremper et al.⁶ said that "a heated skin surface gas tension should not be made to equal an arterial value." Indeed there is no reason to expect that these transcutaneous and intracutaneous sensors should pick up only the gases or pH from the blood of the "arterialized" capillaries; there are oxygen, carbon dioxide, and hydrogen ion in the intracellular and intercellular tissues as well as in the vessels. Continuous sensors will pick up not only what comes from the vessels but also what comes from the intracellular and intercellular tissues, since they are in close proximity to them. Specifically, in the cases studied here, we used a depilatory cream instead of shaving the skin, allowing the electrodes to be more intimately connected to the fetal skin because of more thorough hair removal. This may explain why $tcPCO_2$ values reported here are higher than similar ones reported by authors who did not use a depilatory cream.⁷

Even under resting conditions, there will be a gradient of tension for oxygen that is higher in blood and lower in cells⁸ and for carbon dioxide and acids that is higher in the cells and lower in the blood,⁹ especially in view of the buffering capacity of blood. Under pathologic and stressful physiologic conditions of hypoxemia, redistribution of fetal cardiac output occurs. There is preferential perfusion of vital organs (brain, myocardium, and adrenals), while skin perfusion is decreased secondary to profound catecholamine-mediated vasoconstriction.¹⁰

The gradients will be even steeper since decreased skin perfusion leads to locally lower pH and PO_2 as well as higher PCO_2 while corresponding central blood values change less because of redistribution of regional

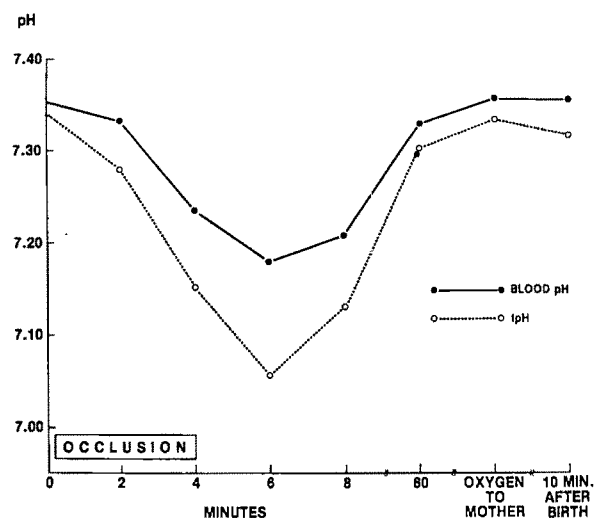
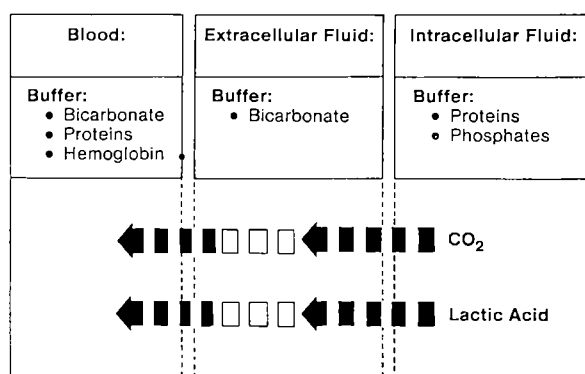


Fig. 5. Four-minute maternal aortic occlusion. Before and late after occlusion, tpH was barely lower than blood pH. During and immediately after occlusion, tpH is much lower. Under stress the difference in hydrogen ion concentration between cells and blood was much greater, which was primarily a result of the greater buffering capacity of blood (see Fig. 6). Figs. 4 and 5 are symmetrically opposite but otherwise identical.

blood flow. Consequently, there will be considerably less oxygen in the cells than in the blood and even more carbon dioxide in the cells than in the blood. The intercellular tissue occupies an intermediary position.¹¹ Hydrogen ions, accumulated in the skin and muscle (along with blood that is lower in PO_2 and higher in PCO_2 from these tissues), will be admixed with the central circulation after the hypoxemic episode has ended, leading to further asphyxial changes in these values before recovery occurs.

If $tcPCO_2$ is higher than blood PCO_2 , it does not mean that the former is incorrectly registered from blood and that the latter is correctly registered from blood. Rather, PCO_2 is higher in tissues than in blood, carbon dioxide is not completely transferred from cells to blood, and, as carbon dioxide arrives in blood, it is buffered. The same reasoning may be applied to oxygen, except that excess PO_2 is not buffered in blood and can therefore be quickly moved to the cells. In other words, tissue (by this we mean intracellular and intercellular tissues) PCO_2 will always be higher than blood PCO_2 , while blood PO_2 and pH will always be higher than tissue PO_2 and pH; ΔPO_2 , ΔPCO_2 , and ΔpH will always be greater under stress and under pathologic conditions than at physiologic rest. The animal studies presented here seem to demonstrate these physiologic principles well. It is not logical to have a "correction factor" when PO_2 , PCO_2 , and pH are measured from different compartments of the body (Fig. 6). Transcutaneous and intracutaneous monitoring have their own physiologic features that are related to but are



From a conceptual and clinical point of view one may argue about the value of data derived from cutaneous tissue as compared with other more critical organ systems and/or central blood parameters. However, the former is considerably more amenable to measurement and in this study seems to very closely reflect and even precede trends taking place in the central circulation. Furthermore, close correlation between fetal scalp blood gas measurements with centrally derived arterial and venous values has been demonstrated by other investigators using animal models under both baseline and stressful conditions¹² as well as in humans.¹³ Last, the fetal scalp pH taken late in labor seems to closely relate to immediate neonatal condition as judged by Apgar scores.^{14, 15} It would therefore seem that other related data derived from cutaneous tissue may likewise be of clinical value and importance.

At present continuous tissue monitoring is not ready to replace intermittent hematologic monitoring for several reasons. Very little is known about the physiology of pH, PO_2 , and PCO_2 at the tissue level, in the intracellular compartment as well as in the intercellular compartment.¹⁶ As well, interpretation of the data from continuous monitoring is difficult, and there seems to be a greater familiarity with hematologic information. It will be only with extensive experimentation on the animal fetus that more knowledge will be gained, for a tremendous variety of pathologic conditions such as severe hypoxia or hyperoxygenation can be produced and investigated. Present technology, while making progress, does not yet allow an easy implantation of the continuous pH, PO_2 , and PCO_2 electrodes in the human fetus.^{3, 1} Clinicians still prefer intermittent fetal scalp blood sampling rather than continuous monitoring. Limited experience with the latter in human fetuses has been noteworthy for drift that increases with the duration of the implantation, frequent detachment of the electrodes, and difficulties in interpreting the results.^{3,7} Consequently, continuous gas and acid-base monitoring remains an excellent research tool, which hopefully will find future clinical application.

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Expression of trophoblast-leukocyte common antigens and placental-type alkaline phosphatase in complete molar pregnancy

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The current study was undertaken to determine the localization of trophoblast-leukocyte common antigens and placental-type alkaline phosphatase in complete molar pregnancy with the use of rabbit antiserum and murine monoclonal antibodies in immunofluorescence assays. Trophoblast-leukocyte common antigens were expressed on all normal villous trophoblast (8 to 38 weeks' gestation) and on all complete moles studied. Placental-type alkaline phosphatase was not expressed on villous trophoblast before 20 weeks' gestation in either normal placentas or complete moles. In contrast, there was strong expression of placental-type alkaline phosphatase on villous trophoblast of normal placentas of more than 20 weeks' gestational age. The expression of polymorphic antigens such as trophoblast-leukocyte common antigens and placental-type alkaline phosphatase on molar trophoblast may be important in the immunobiologic features of gestational trophoblastic disease. (*AM J OBSTET GYNECOL* 1986;155:443-6.)

Key words: Trophoblast-leukocyte common antigens, placental-type alkaline phosphatase, mole

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Several human trophoblast-associated membrane antigens have been characterized in recent years with the use of rabbit polyclonal antisera and murine monoclonal antibodies and have been used to define trophoblast subpopulations in the human placenta and placental bed.^{1,2} Faulk and McIntyre¹ have identified a trophoblast-leukocyte common antigen system (termed TLX antigens) through the use of rabbit antitropho-

Table I. Reactivity of antibodies to TLX antigens (anti-TLX 5, H316) and placental-type alkaline phosphatase (H317) with normal placenta and complete mole in immunofluorescence

<i>Trophoblast</i>	<i>Anti-TLX 5</i>	<i>H316</i>	<i>H317</i>
Normal placental trophoblast			
>20 Weeks' gestation	+++	+	++
<20 Weeks' gestation	+++	+	-
Complete molar trophoblast, <20 Weeks' gestation	+++	+	-

blast sera and have proposed that TLX antigens may be important in immunoregulatory events underlying normal maternal-fetal interactions. Johnson et al.² have produced and characterized several murine monoclonal antibodies raised against syncytiotrophoblast plasma membrane preparations from human term placenta. One such antibody, H316, predominantly reacts with all peripheral blood leukocytes, certain epithelia, and all trophoblast populations throughout pregnancy.^{2,3} The distribution of reactivity of H316 has suggested the possibility that its specificity could be related to the TLX antigen system. Another monoclonal antibody (H317) reacts specifically with the placental-type alkaline phosphatase isoenzyme, which appears in syncytiotrophoblast and some cytotrophoblast after mid-gestation.^{3,4} Both H316 and H317 react with the BeWo human gestational choriocarcinoma cell line, with a number of human transformed cell lines, and with cell lines established from cancer of the larynx, cervix, and breast.¹ The antigens recognized by H316 and H317 can therefore be categorized as oncofetal antigens.

The current study was undertaken to determine the localization of TLX antigens and placental-type alkaline phosphatase in complete hydatidiform mole. It is still unclear where complete mole fits into the spectrum between a variant of missed abortion and a true trophoblastic neoplasm. Investigation of the expression of these antigen systems in complete mole contributes to the body of knowledge concerning the immunobiologic characteristics of gestational trophoblastic disease and the relationship between complete hydatidiform mole and normal placenta.

Material and methods

Tissue specimens. Fresh tissue was obtained from eight normal placentas from the first (8, 10, and 11 weeks' gestation), second (15 and 20 weeks' gestation), and third (38 weeks' gestation) trimesters at the time of therapeutic abortion or term delivery. Tissue from complete hydatidiform mole was obtained by suction curettage from seven patients. After suction curettage,

one patient developed locally invasive gestational trophoblastic tumor and was treated by hysterectomy and adjuvant chemotherapy.

Placental and molar tissue pieces were washed in phosphate-buffered saline to reduce blood contaminants, snap frozen in liquid nitrogen, embedded in Optimum Cutting Temperature Compound (Lab-Tek Products, Naperville, Illinois), and sectioned at 6 μ m. One section from each tissue sample was fixed in cold methanol and stained with hematoxylin and eosin. The remaining frozen sections were fixed in cold methanol for 10 minutes and stored sealed at -70° C until used for immunofluorescence studies.

Antisera. The production and tissue reactivity of the absorbed rabbit antiserum that was reactive with TLX antigen (anti-TLX 5) have been described previously.¹

The production and tissue reactivity of the murine monoclonal antibodies (H316 and H317) have been described in previous reports.²⁻⁴ Spent hybridoma cell culture supernatants were used in this study.

Indirect immunofluorescence assay. Methanol-fixed 6 μ m cryostat sections of eight normal chorionic villous tissues from 8 to 38 weeks' gestation and seven complete hydatidiform moles were evaluated for TLX and placental-type alkaline phosphatase antigen expression by indirect immunofluorescence. Anti-TLX 5, H316, and H317 were diluted 1:100, 1:50, and 1:50, respectively, in phosphate-buffered saline containing 1% bovine serum albumin that had been centrifuged immediately beforehand at 15,000 $\times$ g for 10 minutes to remove aggregates. Fluorescein isothiocyanate-labeled goat antirabbit and goat antimouse IgG (Cappel Laboratories, Cochranville, Pennsylvania) were diluted 1:40 in phosphate-buffered saline-bovine serum albumin. The technique for performing indirect immunofluorescence on fresh-frozen tissue sections has been described previously.^{5,6}

Results

The reactivity of anti-TLX 5, H316, and H317 with normal placental villous tissue and complete hydatidiform mole is presented in Table I. Anti-TLX 5 and H316 were both strongly reactive with all villous trophoblast of the seven complete moles tested and with all normal placental trophoblast from 8 to 38 weeks' gestation. As reported previously, H317 was reactive with villous syncytiotrophoblast on its apical surface in placentas that were 20 weeks' gestational age or older but was not reactive with villous trophoblast from younger placentas (<20 weeks) (Fig. 1).³ H317 was not reactive with villous trophoblast from any of the seven complete moles (gestational ages ranging from 10 to 15 weeks), including molar villous trophoblast from one patient who subsequently developed persistent non-metastatic gestational trophoblastic tumor.

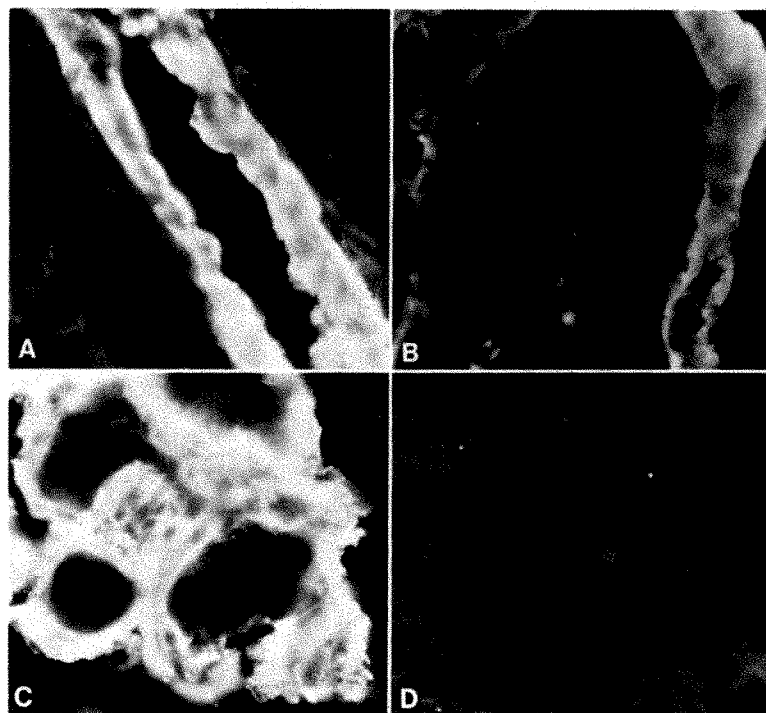


Fig. 1. A, Photomicrograph of chorionic villi from an 11-week placenta showing expression of H316 by villous trophoblast in an immunofluorescence assay. (Original magnification $\times 1300$.) B, Photomicrograph of molar chorionic villi showing expression of H316 by villous trophoblast in an immunofluorescence assay. (Original magnification $\times 1300$.) C, Photomicrograph of chorionic villi from a 38-week placenta showing expression of H317 by villous trophoblast in an immunofluorescence assay. (Original magnification $\times 1300$.) D, In this photomicrograph of molar chorionic villi, the villous trophoblast shows no reactivity with anti-H317 antibody in an immunofluorescence assay. (Original magnification $\times 1300$.)

Comment

Immunohistologic studies have indicated that molar trophoblast is more akin to normal placental trophoblast than choriocarcinoma in the expression of certain cellular products. For example, both molar and placental villous syncytiotrophoblast express α_2 -macroglobulin, placental protein 5, and certain characteristic trophoblast membrane antigens, whereas these molecular species are undetectable in choriocarcinoma.^{7,9} We have also recently compared the expression of murine stage-specific embryonic antigens and human sperm antigens in normal placenta, hydatidiform mole, and choriocarcinoma.^{6, 10} In studies to date, molar villous trophoblast has shown the same reactivity with monoclonal antibodies to oncofetal antigens as normal villous trophoblast.

In this study, molar villous trophoblast also demonstrated immunohistologic reactivity similar to that of normal villous trophoblast with antibodies directed against TLX antigens and placental-type alkaline phosphatase. TLX antigens, as detected by a rabbit antiserum (anti-TLX 5) and a murine monoclonal antibody (H316), were expressed on all normal villous trophoblast (8 to 38 weeks' gestation) and all complete moles

studied. Placental-type alkaline phosphatase, as detected by a murine monoclonal antibody (H317), was not expressed on villous trophoblast before 20 weeks' gestation in either normal placentas or complete moles. In contrast, there was strong expression of placental-type alkaline phosphatase on villous syncytiotrophoblast of normal placentas of more than 20 weeks' gestational age. Complete hydatidiform molar tissue of 20 weeks' gestational age or more was not available, since patients with complete mole generally present before the twentieth week of gestation. Data from the current study thus continue to support the concept that molar trophoblast is like normal placental trophoblast in the expression of oncofetal antigens.

Both TLX and placental-type alkaline phosphatase are polymorphic antigen systems.^{3, 11} Placental-type alkaline phosphatase may be an important cell surface modulator of trophoblastic differentiation and growth after midpregnancy by acting on protein phosphotyrosine at physiologic pH³; tyrosine phosphorylation is a key event in the control of cell growth.¹² Molar villous trophoblast, like placental villous trophoblast, does not express placental-type alkaline phosphatase before 20 weeks' gestation. These data suggest a similarity of

growth-regulatory controls of normal early placental and molar trophoblast.

The TLX antigen system has been attributed a central role in regulating maternal-fetal immunologic interactions within placental and uterine tissues.^{1,13} Since the TLX antigen system is polymorphic, paternal TLX antigens expressed on trophoblast could often be expected to elicit a maternal immune response.¹¹ Faulk et al.¹⁴ have postulated that normal pregnancy requires the maternal production of anti-TLX antibodies that serve as blocking antibodies to protect the fetal graft from immunologic rejection. Patients with unexplained recurrent spontaneous abortion reportedly lack blocking antibodies, and these couples might often share similar TLX antigen profiles.^{1,11} Maternal response to TLX antigens could also take the form of active recruitment of suppressor lymphocytes into local lymph nodes and decidual tissues.¹

The expression of polymorphic antigens such as TLX and placental-type alkaline phosphatase on molar trophoblast may be particularly important in the immunobiologic features of gestational trophoblastic disease. Because all chromosomes are of paternal origin in complete hydatidiform mole, this tissue is a complete allograft.¹⁵ Complete hydatidiform mole might therefore be expected to induce a vigorous maternal response, which may in part serve to protect the molar allograft from immunologic rejection. Survival of the molar allograft or gestational trophoblastic tumor could be supported by maternal response to these antigens with production of blocking antibody and active recruitment of suppressor lymphocytes. The pathogenesis of molar pregnancy and gestational trophoblastic disease would thus be influenced by various competing immunologic interactions between the maternal host and molar tissue. Further understanding of the immunobiologic characteristics may enable the identification of aggressive molar pregnancies and gestational trophoblastic tumors that require intensive hormonal follow-up and chemotherapy and may also enable the development of immunotherapy for selected patients who have rapidly progressive or drug-resistant tumors.

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Follicle-stimulating hormone and estrogen elevate deoxyribonucleic acid α -nucleotidyltransferase activity in relationship to deoxyribonucleic acid synthesis in immature rat ovaries

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The deoxyribonucleic acid polymerase activities and deoxyribonucleic acid contents of ovaries were measured to clarify the relationship between hormone-stimulated cell proliferation and the enzymes in ovaries of immature intact rats (4 to 29 days after birth) and hypophysectomized rats. The specific activity of deoxyribonucleic acid α -nucleotidyltransferase (the activity per microgram of deoxyribonucleic acid of the ovaries) drastically increased with an increase in the deoxyribonucleic acid content of the ovaries from 4 to 14 days after birth and then remained constant or slightly decreased after the increase, while the activity decreased gradually after hypophysectomy with no increase in the DNA content. Ovine follicle-stimulating hormone or estradiol-17 β enhanced the deoxyribonucleic acid synthesis and α -nucleotidyltransferase activity in the ovaries of hypophysectomized rats, while deoxyribonucleic acid β -nucleotidyltransferase activity showed no significant change. Ovine luteinizing hormone, progesterone, and testosterone caused no significant increases. These results suggest that follicle-stimulating hormone or estrogen causes the induction of deoxyribonucleic acid α -nucleotidyltransferase accompanied by deoxyribonucleic acid synthesis in cell proliferation in immature rat ovaries. (AM J OBSTET GYNECOL 1986;155:447-51.)

Key words: Deoxyribonucleic acid α -nucleotidyltransferase, follicle-stimulating hormone, estrogen, ovarian follicles

Follicle-stimulating hormone (FSH) has been reported to increase the deoxyribonucleic acid (DNA) content of rat ovaries¹ and to enhance the incorporation of radioactive thymidine into DNA and cell division in ovaries.² Estrogen is also considered to stimulate DNA synthesis and cell division in immature rat ovaries.²⁻⁴ These reports indicate that FSH or estrogen participates in follicle growth by stimulating DNA replication and then cell division in the ovaries. However, very few studies showing further metabolic pathways leading to DNA replication have been reported.

Three species of DNA nucleotidyltransferase (α , β , and γ) have been found and characterized in eukaryotes including the rat.⁵ DNA α -nucleotidyltransferase is thought to participate in nuclear DNA replication.⁶ The function of DNA β -nucleotidyltransferase is not known but it is tentatively considered to be related to

DNA repair.⁶ DNA γ -nucleotidyltransferase is localized in the mitochondria and causes the replication of mitochondrial DNA.⁶

Recently, possible relationships between hormones and DNA nucleotidyltransferase have been reported in the rat uterus,⁷ adrenal glands,⁸ testis,⁹ and oocyte.¹⁰

In the present study, we examined the relationship between FSH- or estrogen-stimulated DNA synthesis and DNA α - and β -nucleotidyltransferase activities in immature rat ovaries.

Material and methods

Immature female Wistar-Imamichi rats, obtained from the Imamichi Institute for Animal Reproduction (Saitama, Japan), were used and maintained in an environmentally controlled room illuminated daily from 8:00 AM until 8:00 PM. The rats were divided into the following experimental groups. Group 1 consisted of 4-, 7-, 10-, 14-, 21- and 28-day-old intact rats. Group 2 consisted of rats hypophysectomized at 21 days of age via the parapharyngeal approach, and this group was further divided into two groups: (1) untreated 21-, 24-, 27-, 30-, and 32-day-old hypophysectomized rats and (2) hypophysectomized rats treated daily with var-

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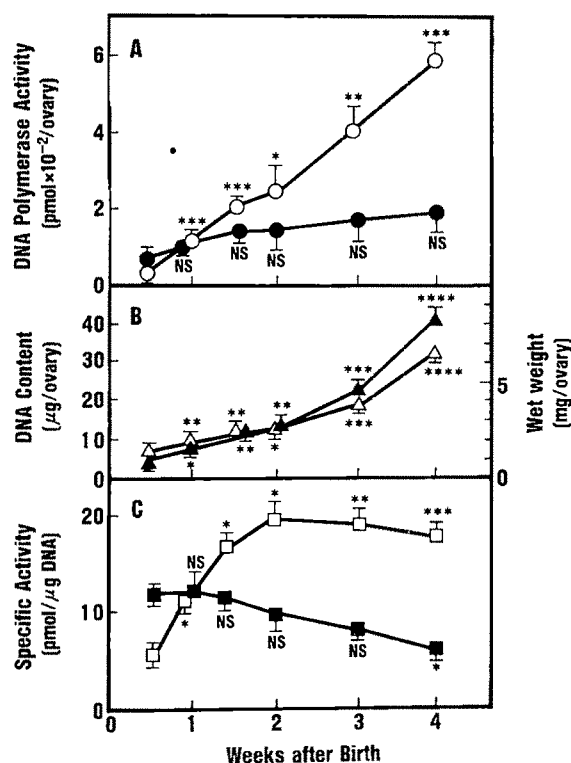


Fig. 1. Changes in DNA nucleotidyltransferase (*polymerase*) activity (A), DNA content (B), and specific activity (C) in immature rat ovaries. $\circ$, DNA α -nucleotidyltransferase activity; $\bullet$, DNA β -nucleotidyltransferase activity; Δ , DNA content; $\blacktriangle$, wet weight; $\square$, specific activity of DNA α -nucleotidyltransferase; and $\blacksquare$, specific activity of DNA β -nucleotidyltransferase. Each value is the mean $\pm$ SE (vertical bars) for four animals. Significance of differences versus 4-day-old rats: *, $p < 0.05$; **, $p < 0.02$; ***, $p < 0.01$; ****, $p < 0.001$; NS, not significant.

ious hormones beginning at 25 days of age, that is, 4 days after hypophysectomy, and continuing until autopsy at 29 days of age (unless otherwise stated). In group 1, the intact rats were decapitated while under ether anesthesia at 4, 7, 10, 14, 21, and 28 days of age. In group 2, hypophysectomized rats were treated daily with FSH (2 μ g/day in saline solution subcutaneously), luteinizing hormone (LH) (2 μ g/day in saline subcutaneously), estradiol (E_2) (1 mg/day in sesame oil subcutaneously), progesterone (1 mg/day in sesame oil subcutaneously), or testosterone (1 mg/day in sesame oil subcutaneously), respectively, for 4 days before autopsy at 29 days of age. Untreated hypophysectomized rats were decapitated at 0, 3, 6, 9, and 11 days after the hypophysectomy. After autopsy, the ovaries were quickly removed and the ovarian wet weights were determined after removal of the oviduct and surrounding tissues. The ovaries then were used for the experiments.

FSH (ovine FSH, oFSH-15, AFP-5529C, from the National Institute of Arthritis, Metabolism, and Digestive Diseases having an FSH potency 20 times that of FSH-S1 from the National Institutes of Health, an LH potency 0.04 times that of LH-S1 from the National Institutes of Health, and a prolactin potency less than 0.001 times that of prolactin from the National Institutes of Health) and LH (ovine LH, oLH-24, AFP-0754 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, having an LH potency 2.3 times that of LH-S1 from the National Institutes of Health, an FSH potency less than 0.005 times that of FSH-S1 from the National Institutes of Health, and a prolactin potency less than 0.001 times that of prolactin from the National Institutes of Health) were gifts from Dr. S. Raiti. E_2 , progesterone, and testosterone were obtained from Sigma Chemical Co. (St. Louis, Missouri). To measure DNA nucleotidyltransferase activities, one to five ovaries were homogenized in 100 to 200 μ l (100 μ l/5 mg wet weight of ovary) of 1.0% Triton X-100 sucrose (0.25 mol/L), potassium phosphate buffer (20 mmol/L; pH 7.9) ethylenediaminetetraacetic acid (5 mmol/L), 2-mercaptoethanol (5 mmol/L), sodium chloride (0.5 mol/L), and bovine serum albumin (1 mg/ml) with a Teflon-glass homogenizer, followed by centrifugation at $10,500 \times g$ for 30 minutes. After the centrifugation, 50 μ l of the supernatant was mixed with 25 μ l of 90% glycerol. Aliquots (0.5 to 2.0 μ l) of the supernatant were assayed for DNA nucleotidyltransferase activities according to our previous method.^{10,11} DNA polymerase activity was expressed as picomoles of deoxynucleotide 5'-monophosphate incorporated in 20 minutes at 37° C.

Furthermore, in order to measure the DNA content of the ovaries, the ovaries were treated and centrifuged as described for the measurement of DNA nucleotidyltransferase activities. After the centrifugation, the precipitate suspended in 100 μ l of Tris hydrochloride buffer (25 mmol/L, pH 7.5) was reprecipitated by adding 900 μ l of 10% perchloric acid, followed by centrifugation at $2000 \times g$ for 5 minutes. The precipitate was washed with 5% perchloric acid three times by centrifugation at $2000 \times g$ for 5 minutes. The washed precipitate was assayed for the amount of DNA according to the method of Burton.¹²

Student's t test was used for the statistical analysis.

Results

DNA α -nucleotidyltransferase activity increased linearly from 4 to 28 days after birth, while the β -nucleotidyltransferase activity showed only a slight increase (Fig. 1, A). The DNA content of ovaries increased exponentially during this period (Fig. 1, B). As summarized in Fig. 1, C, the specific activity of DNA

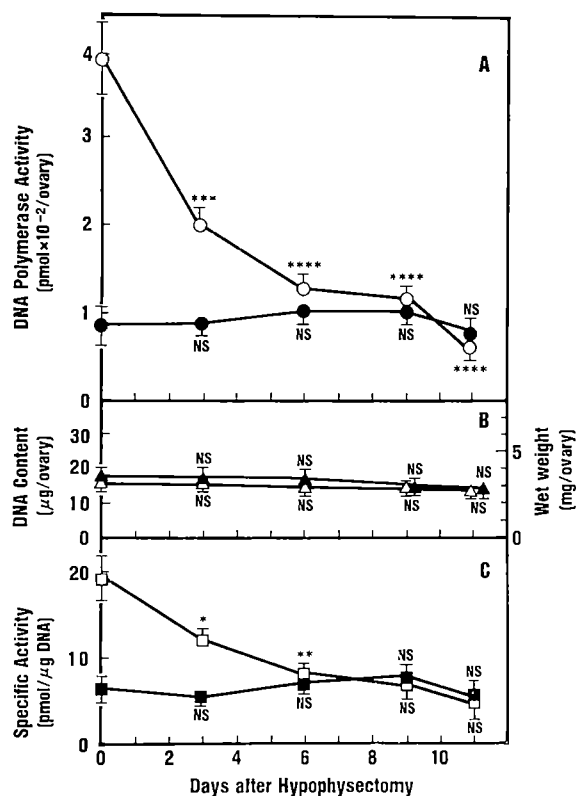


Fig. 2. Changes in DNA nucleotidyltransferase (*polymerase*) activity (A), DNA content (B), and specific activity (C) after hypophysectomy in immature rat ovaries. ○, DNA α-nucleotidyltransferase activity; ●, DNA β-nucleotidyltransferase activity; △, DNA content; ▲, wet weight; □, specific activity of DNA α-nucleotidyltransferase; and ■, specific activity of DNA β-nucleotidyltransferase. Each value is the mean ± SE (vertical bars) for four animals. Significance of differences versus day 0 rats, after hypophysectomy (25-day-old intact rats): *, $p < 0.05$; **, $p < 0.02$; ***, $p < 0.01$; ****, $p < 0.001$; NS, not significant.

α-nucleotidyltransferase (DNA nucleotidyltransferase activity per microgram of ovarian DNA) increased drastically from 4 to 14 days after birth and thereafter remained constant or slightly decreased, whereas the specific activity of DNA β-nucleotidyltransferase decreased gradually from 4 to 28 days after birth.

In hypophysectomized rat ovaries, no significant increase in the DNA content was detected during the 10 days after hypophysectomy (Fig. 2, B). DNA α-nucleotidyltransferase activity began to decrease after hypophysectomy and leveled off at 6 days after hypophysectomy (Fig. 2, A). The specific activity of DNA α-nucleotidyltransferase showed a similar decrease (Fig. 2, C). DNA β-nucleotidyltransferase activity did not change significantly (Fig. 2, A and C).

The effects of hormones on the enhancement of DNA synthesis and DNA nucleotidyltransferase activities were examined in hypophysectomized rat ovaries

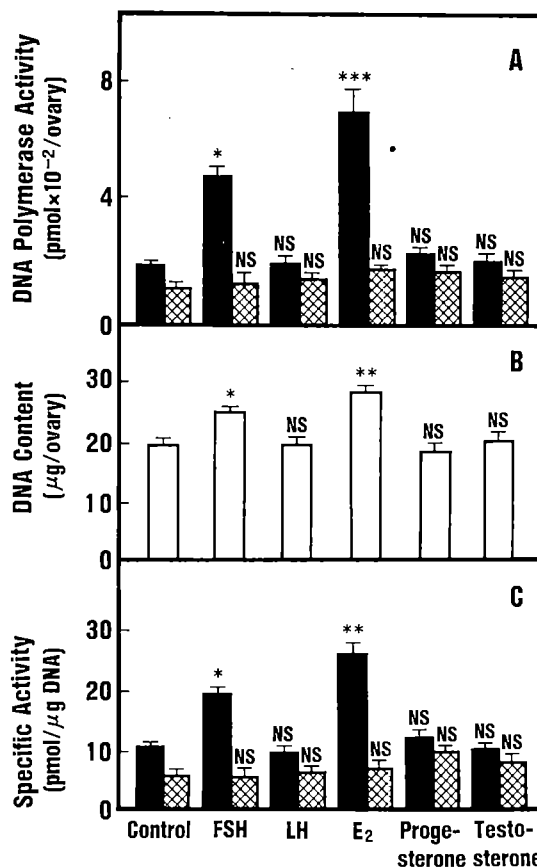


Fig. 3. Enhancement of DNA nucleotidyltransferase (*polymerase*) activity (A), DNA content (B), and specific activity (C) with hormones in hypophysectomized rat ovaries. Hypophysectomized rats were injected daily with FSH (2 μg/day), LH (2 μg/day), E₂ (1 mg/day), progesterone (1 mg/day), or testosterone (1 mg/day) as described in Materials and methods. Sesame oil was injected as a control. Five days after the injections were begun, DNA nucleotidyltransferase activities and DNA content were measured as described in the text. ■, DNA α-nucleotidyltransferase; ▨, DNA β-nucleotidyltransferase; and □, DNA content. Each value is the mean ± SE (vertical bars) for four animals. Significance of difference versus untreated hypophysectomized rats: *, $p < 0.05$; **, $p < 0.02$; ***, $p < 0.01$; NS, not significant.

(Fig. 3). An increase of 5.0 μg of DNA per ovary occurred with the administration of FSH 5 days after the hormone injections were begun. Similar increases in the DNA content were seen in intact rats during the period of 2 to 4 weeks after birth (Fig. 1, A). DNA α-nucleotidyltransferase activity and its specific activity also increased to levels that corresponded to the normal levels in intact rats at FSH injection. However, no significant increase in DNA β-nucleotidyltransferase activity was observed with FSH injection. E₂ also enhanced the DNA synthesis and DNA α-nucleotidyltransferase activity but not DNA β-nucleotidyltransferase activity. The specific activity of DNA α-nucleotidyltransferase

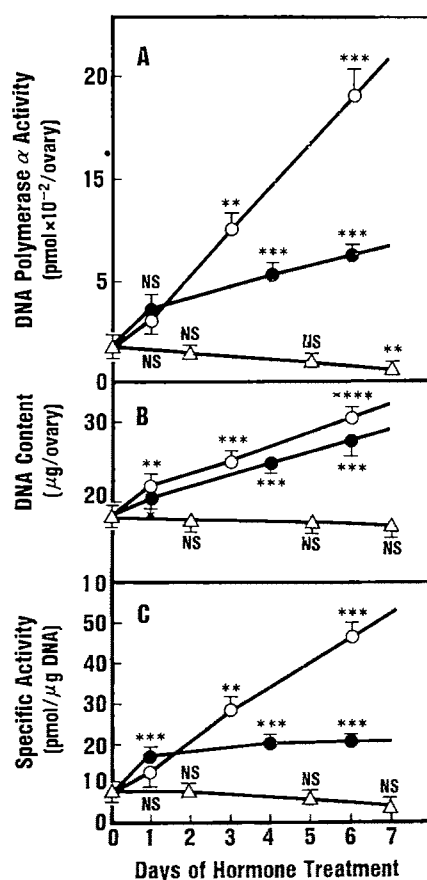


Fig. 4. Time course of enhancement of DNA α -nucleotidyltransferase activity by FSH and E_2 in hypophysectomized rat ovaries. Hypophysectomized rats were injected daily with FSH (2 μ g/day), E_2 (1 mg/day), or sesame oil only (control) as described in the text except that the injections were continued for 1 to 7 days. On the day after the last hormone injection, as indicated in the figure, DNA α -nucleotidyltransferase (polymerase α) activity (A), DNA content (B), and specific activity (C) were measured as described in the text. $\circ$, E_2 -treated hypophysectomized rats; $\bullet$, FSH-treated hypophysectomized rats; and $\triangle$, untreated hypophysectomized rats. Each value is the mean $\pm$ SE (vertical bars) for four animals. Significance of difference versus untreated hypophysectomized 25-day-old rats (4 days after hypophysectomy): *, $p < 0.05$; **, $p < 0.02$; ***, $p < 0.01$; NS, not significant.

increased to 2.4-fold that of untreated hypophysectomized rat ovaries. On the other hand, LH, progesterone, and testosterone caused no significant increases in DNA synthesis and DNA nucleotidyltransferase activities.

Furthermore, the time courses of the enhancement of DNA α -nucleotidyltransferase activity and DNA synthesis by FSH or E_2 treatment were studied in hypophysectomized rats (Fig. 4). DNA α -nucleotidyltransferase activity increased gradually after FSH or E_2 injection, depending on the number of days of hormone treatment, while the activity showed a slight decrease in untreated hypophysectomized rats. The DNA content

also increased gradually after the hormone treatment, depending on the number of days. The rate of increase of the DNA content in the hormone-treated hypophysectomized rats was comparable to that in 3- to 4-week-old intact rats. The specific activity of DNA α -nucleotidyltransferase increased linearly, depending on the number of days of hormone treatment in E_2 -treated hypophysectomized rats. On the other hand, the specific activity increased to a certain level after the initial hormone injection and then remained constant with further daily injections of FSH in FSH-treated hypophysectomized rats. The increased level of the specific activity was quite similar to that in 2- to 3-week-old intact rats. The specific activity showed a slight decrease or remained unchanged in untreated hypophysectomized rats.

The DNA α -nucleotidyltransferase activities were additive and no significant stimulation or inhibition was detected in the ovarian extracts used in this experiment (data not shown).

Comment

Of the activities of DNA α - and β -nucleotidyltransferase, only that of DNA α -nucleotidyltransferase changed in relation to the increase in ovarian DNA content (Figs. 1 to 3). This suggests that DNA α -nucleotidyltransferase participates in DNA synthesis in immature rat ovaries.

In hypophysectomized rat ovaries, DNA α -nucleotidyltransferase activity decreased (Fig. 2), suggesting that the activity is regulated by pituitary gonadotropins. It has been reported that FSH or estrogen enhances DNA synthesis^{1,2,4} but that LH, progesterone, and testosterone cause no significant enhancement² in the growing follicles. In the present study, administration of FSH or E_2 restored the activity in rats (Fig. 3). Therefore, FSH and estrogen seem to enhance the enzyme activity in vivo in immature rat ovaries.

The specific activity of DNA α -nucleotidyltransferase did not change in parallel with the increase in DNA content in the ovaries of intact immature rats (Fig. 1) or hypophysectomized rats (Fig. 2). Therefore it does not seem that DNA α -nucleotidyltransferase activity of the ovaries depends on the number of cells that contain the enzyme; that is, the amount of DNA α -nucleotidyltransferase activity is controlled at the cellular level in the ovaries.

The specific activity of DNA α -nucleotidyltransferase increased from 4 to 14 days after birth in vivo (Fig. 1). Elevation of the concentrations of FSH¹³ and estrogen^{13, 14} in serum have been reported to occur 4 to 15 days after birth in immature female rats, whereas elevations of LH¹⁴ and progesterone^{13, 14} occur at 13 to 16 and 10 to 22 days after birth, respectively. The serum testosterone level is uniformly low in rats.¹⁴ There-

fore, the increase in the specific activity of DNA α -nucleotidyltransferase seems to occur coincidentally with that of serum FSH and estrogen *in vivo*. The specific activity was enhanced by FSH and E_2 treatment in hypophysectomized rats (Figs. 3 and 4). These results indicate that FSH and estrogen induce a high level of DNA α -nucleotidyltransferase activity in the ovaries. Mixing experiments suggested the presence of no stimulators of DNA α -nucleotidyltransferase in the ovaries (unpublished data). Therefore the induced high level of DNA α -nucleotidyltransferase activity seems to be caused by an increase in the DNA α -nucleotidyltransferase molecule itself. From these considerations, we conclude that FSH and estrogen cause the induction of DNA α -nucleotidyltransferase accompanied by DNA synthesis in cell proliferations in immature rat ovaries. Since DNA β -nucleotidyltransferase was not induced by these, the induction seems to be specific. These results suggest that the enzymes that participate in DNA replication are induced.

E_2 caused the induction of DNA α -nucleotidyltransferase in a time-dependent manner, but FSH caused limited induction (Fig. 4). This may suggest that there are different mechanisms of induction for estrogen and FSH. However, further studies are required to clarify this. Fig. 4 also shows that E_2 is more effective than FSH for the induction of DNA α -nucleotidyltransferase. This result may help explain why estrogen is more effective than FSH in granulosa cell proliferation in immature hypophysectomized rat ovaries.⁴

The present study was carried out with ovarian follicles; therefore it may be difficult to define which ovarian cells were responsible for the induction of DNA α -nucleotidyltransferase. However, E_2 and FSH markedly stimulate granulosa cell proliferation in hypophysectomized and intact rats, and the former is more effective than the latter.¹⁵ Furthermore, DNA synthesis due to E_2 or FSH is consistent with granulosa cell proliferation in these cases (unpublished data). This suggests that granulosa cells may be responsible for the induction of DNA α -nucleotidyltransferase.

Recently we reported that DNA α -nucleotidyltransferase is induced and stored in the oocytes of rat preovulatory follicles with FSH or LH.¹⁶ This induction seems to be required for rapid DNA synthesis after fertilization. Accordingly, this report seems to be essentially consistent with the present report in regard to DNA synthesis. DNA β -nucleotidyltransferase has been reported to be regulated by adrenocorticotrophic hormone in the rat adrenal gland⁸ and by testosterone and pituitary gonadotropins in the rat testis.⁹ In these cases, the hormones and DNA β -nucleotidyltransferase are considered to be related to cell differentiation rather than cell proliferation. In the immature rat uterus, estrogen has been reported to enhance DNA synthesis

and cytoplasmic DNA nucleotidyltransferase (corresponding to DNA α -nucleotidyltransferase) activity.⁷ DNA synthesis and DNA α -nucleotidyltransferase activity have also been reported to increase in Leydig cells of diethylstilbestrol-stimulated mouse testes.¹⁶ Therefore, a similar mechanism of estrogen-induced DNA synthesis may be involved in the induction of DNA α -nucleotidyltransferase.

We thank Dr. S. Raiti, University of Maryland, Baltimore, Maryland, for providing us with the ovine FSH and LH.

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Predictive value of ultrasonic targeted imaging for fetal anomalies

To the Editors:

I would like to draw your attention to the article entitled "Predictive value, sensitivity, and specificity of ultrasonic targeted imaging for fetal anomalies in gravid women at high risk for birth defects" by Sabbagha et al. (AM J OBSTET GYNECOL 1985;152:822-7) with regard to the following points: (1) In the abstract there is mention of 569 fetuses whereas in the text the corresponding number mentioned is 596. (2) The authors, while analyzing their data, have wrongly equated the sensitivity to predictive value of abnormal result and the specificity to predictive value of normal result. For correct analyses they need to refer to references 1 to 3. For example, if one refers to Table II on page 824, the correct analysis would have been as follows: sensitivity = 78 of 81 or 96.3% and specificity = 509 of 513 or 99.2%.

Similar corrections need to be made for the corresponding figures stated for sensitivity and specificity on page 825 in the cases of spina bifida, urinary system anomalies, gastrointestinal system anomalies, and skeletal system anomalies.

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Reply

To the Editors:

The correct number of fetuses examined is 596; since two were deleted, the total number used in calculating predictive value, sensitivity, and specificity statistics is 594, as shown in Table II, page 824.

There is a typographic error in Table II because the sensitivity is indeed 96.3% and the specificity is 99.2%.

The data presented in Table IV and the predictive values reported for gastrourinary and gastrointestinal anomalies (derived from Table I), all on page 825, are correct.

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Intrapartum fetal surveillance and fetal scalp blood sampling

To the Editors:

An article by Clark and Paul (AM J OBSTET GYNECOL 1985;153:717-20) supports the proposition that "the properly trained clinician may pursue an approach for the detection of fetal distress that does not include scalp blood sampling without either compromising his ability to detect fetal distress or significantly increase the cesarean section rate." This appeared in the JOURNAL approximately 18 months after an article by Perkins (AM J OBSTET GYNECOL 1984;149:327-36), which concluded that "absence of pH studies did not appear to result in extraordinary rates of stillbirth, operative intervention, or neonatal compromise." I was surprised to find that Drs. Clark and Paul did not include in their discussion or in their list of references the publication by Dr. Perkins. While it is true that Dr. Perkins reviewed the experience of the obstetric service at the University of New Mexico from a slightly different perspective, he arrived at a similar conclusion. The Perkins study was one of the first, if not the first, study to seriously question the value of performing intrapartum fetal pH measurements for clinical management.

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Reply

To the Editors:

In no way did we intend to slight or neglect Dr. Perkins' excellent paper. He has long been an advocate of the position we expressed. At the time of the initial manuscript preparation, Dr. Perkins' article had not appeared. As we were limited to 16 references, we did not add it before final publication.

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Brain stem evoked response and neonatal neurological maturation

To the Editors:

In a recent letter entitled "Effects of hypertension in pregnancy on neurological maturation in neonates" (AM J OBSTET GYNECOL 1985;153:709-10), Diven et al. suggested that maternal hypertension has no effect on infant neurological maturation as measured by wave V latency of the brain stem evoked response. I would like to draw attention to the following points regarding their methodology:

1. In Table I the authors assigned neurological age

in a series of term as well as preterm neonates using Hecox's normative data.¹ Unfortunately the reference used does not provide this information. Hecox and Galambos,¹ who used auditory evoked responses and estimated its usefulness in assessing hearing in infants and adults, have not described criteria for neurological age assignment in preterm neonates by this method. There were only three preterm neonates in Hecox's study who were examined at the age of 3 weeks or beyond, but no neurological age was assigned to them. It is puzzling that without having a standard norm for neurological age assignment, the authors determined the age on a weekly basis, that is, 33 weeks, 34.5 weeks, etc.

2. Gould et al.² defined accelerated neurological maturation by clinical examination of the neonates as neurological age of 3 weeks in advance of the reported gestational age. Three weeks was chosen because this fell well outside Dubowitz's 95% confidence limit. Anatomically, acceleration of fetal cerebral maturation was also defined by Hadi³ as enhancement of maturation in excess of 2 weeks beyond gestational age estimated by menstrual period and/or early ultrasound examination. Acceleration of >2 weeks was chosen because every 2 to 3 weeks a number of additional cerebral gyri, sulci, depths, or sinuses appear, which represent a given gestational age in preterm neonates.

The authors concluded from their data that only one neonate (No. 5) who had neurological acceleration of >2 SD (21 weeks greater than clinical gestational age) was considered significant, and those with neurological enhancement of 5 weeks, 6 weeks, or 7 weeks were interpreted as insignificant. The authors did not provide basis for definition of acceleration of maturation by their method. I believe use of a score of >2 SD in this matter is unreasonable.

Brain stem auditory evoked response can provide an objective useful method for assessing hearing in infants and adults. Normative data regarding neurological age assignment in preterm neonates by this method is lacking at the present time. Therefore assessment of neurological acceleration in preterm neonates without a standard norm is not possible. Considering perinatal clinical implication, I disagree with the authors that acceleration of maturation in excess of 5, 6, or 7 weeks are not significant.

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Reply

To the Editors:

We are thankful that Dr. Hadi in his letter has pointed out an inaccuracy regarding a reference that we had cited. The latency values to which we compared both our study group and control group were taken from our auditory brain stem response form which is used in administering hearing tests on newborn infants, many of whom are preterm. This form was derived in part from the report of Hecox and Galambos¹ as to how auditory brain stem response values vary with decibel level of the stimulus; however, the actual latency values for gestational age were derived from normative data collected at our own institution. We regret our oversight in failing to state such in our original letter. As our study was a pilot study of sorts, we discovered that use of normative data is a major factor if the auditory brain stem response is to be used in assessing neurological age. One method is to use one's own institutional norms. Another approach is to compare absolute latencies with those of age-matched control subjects as was done by Henderson-Smart et al.²

Regarding the second point, although one might deduce that an acceleration of neurological maturation in excess of 2 or 3 weeks is clinically or anatomically significant, what we reported was statistical significance. The probability of a certain measured difference being significant is unique to each set of data collected. Dubowitz et al.³ found +2 weeks to be outside the 95% confidence limits. Gould et al.⁴ arbitrarily defined accelerated neurological maturation as neurological age 3 weeks in advance of gestational age with use of Dubowitz's data although their own data had a similar value of +4 weeks. We could not use previously determined values for assigning significant neurological maturation because our method of assessing neurological age was altogether different from the methods of Dubowitz et al. and Gould et al. The fact that our study group was small also made it more likely that a greater difference between neurological and gestational age would be required to be statistically significant.

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Electronic fetal monitoring as seen through the looking glass

To the Editors:

I was reading the December 1, 1985, issue of the *AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY* during the commercial breaks of the television show, *Alice in Wonderland*. Two papers (Clark SL, Paul RH. Intrapartum fetal surveillance: The role of fetal scalp blood sampling. 1985;153:717-20, and Keegan KA, Waffarn F, Quilligan EJ. Obstetric characteristics in fetal heart rate patterns of infants who convulsed during the newborn period. 1985;153:732-7) indicated to me how appropriate the classic Lewis Carroll's spoofs are for some of our clinical comments. As Humpty Dumpty stated, "When I use a word, it means just what I choose it to mean. Neither more or less." Alice asked in return, "The question is whether you can make words mean so many different things?"

For instance, I thought that the data of Keegan et al. showed quite clearly that electronic fetal monitoring in a very large, well-managed series did not necessarily prevent neonatal seizures and subsequent cerebral palsy. The fetal heart rate (FHR) patterns of the newborn infants who convulse were either not abnormal by current criteria or the occurrence of the abnormal fetal heart rate pattern was of such short duration that there was insufficient time for effective obstetric intervention. I believe that this must be about the most important finding ever made with electronic fetal monitoring and indicates that our present concepts are probably incorrect. Instead, the authors state that "their report only indicates that research . . . be continued." The authors may have been thinking of the many prior studies that suggest that electronic fetal monitoring reduces the incidence of cerebral palsy, causing me to recall the Queen's remarks, "It's a poor sort of memory that only works backwards," or perhaps our words do "mean so many different things." I wonder what the

outcomes would have been if fetal scalp sampling had been used more frequently?

However, Clark and Paul's paper suggests that fetal scalp pH assessment be "de-emphasized." They wish to establish a new gold standard which apparently would be fetal heart rate accelerations after fetal scalp stimulation. In contrast, the current trend elsewhere is to collect cord pH and blood gas data for "legal" protection for any case in which the infant may not do well. In other words, umbilical cord pH is still the "gold standard" as an indicator of newborn condition, and this value correlates with the scalp pH.²

Clark and Paul fail to mention the frequent lack of objectivity of interpretation in fetal heart rate monitoring tracings. It has been my experience that in the case of an acidotic newborn infant, there often is disagreement during rounds or in the courts among the experts as to how or even whether the heart rate tracing was abnormal.

Fig. 1 is a recording in a postdate pregnancy patient in labor, 3 cm dilated, and with meconium-stained fluid for which the house officer obtained a fetal scalp pH. I interpreted the fetal heart rate as "not worrisome enough for a scalp pH." However, there were three scalp pH values <7.20, and with immediate delivery by cesarean section the infant had an umbilical artery pH of 7.09. Despite heroic treatment the infant died at <24 hours of age of a combination of meconium aspiration and asphyxia. Admittedly, based on the authors' experience that given "more understanding of fetal heart patterns," this fetal acidemia might have been recognized in the absence of performing a scalp pH.

Concerning Humpty Dumpty's use of words, the authors state "that there is little evidence to justify the assumption that nonacidotic fetus which has lost its capacity for FHR acceleration is in fact not distressed." If I understand these words, their only support is a study related to scalp pH determination by Dr. Clark.³ Indeed, I have been studying fetal responses to stimuli (including sound, vibration, and scalp or head stimulation) since the early 1970s.^{4,5} In my experience the fetal response depends on maternal narcotic levels or fetal state in addition to its well-being. Also, I believe that the authors are confusing the words fetal *distress*

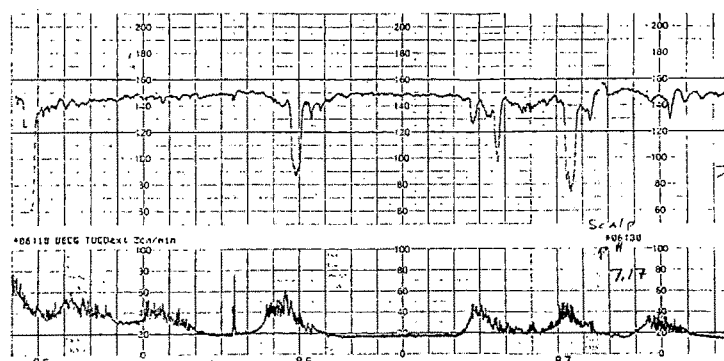


Fig. 1. Fetal heart rate recording immediately before scalp sampling.

with fetal stress, but as pointed out by Humpty Dumpty, they can make the words mean whatever they wish. However, I have tried to distinguish between these two terms.⁶

Finally, it is my impression that in order to legitimately champion electronic fetal monitoring over other techniques would require Clark and Paul to demonstrate that their technique when properly performed is essentially without error. Apparently, Keegan et al., using data from Clark and Paul's own institution, have found that this was not so. Or as the Gryphon remarked, "that's the reason they're called lessons, because they lesson from day to day."

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Reply

To the Editors:

Dr. Goodlin has commented uniquely regarding his interpretation of our recently published article relevant to the role of fetal blood sampling. Dr. Goodlin may have failed to grasp the intent of the paper, which was not to establish a new gold standard but to encourage a more critical and indeed objective evaluation of fetal heart monitoring tracings.

As was stated in Dr. Goodlin's letter, we recognize the confusion that exists, particularly in regard to the definition of abnormal fetal heart rate patterns. Indeed the greatest arena of disagreement exists in defining how "bad is bad" and likewise in the separation of the term fetal distress from that of fetal stress.

Dr. Goodlin has generously supplied an example of a case which is in fact one of the "lessons that we must learn from day to day." In the case example presented the baseline heart rate is at a level of 150 bpm with decreased variability and, to my evaluation, lacks the very accelerations that we have emphasized as being positive predictors.

Finally, the challenge to demonstrate that any technique is essentially without error is an enormous request to be placed on a biologic evaluator.

We are continually indebted to Dr. Goodlin for his

thoughtful and provocative critique of the words that we use. Perhaps we will all benefit from the case example that he has provided in that we may all learn a new lesson.

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Dystocia, parity, and the cesarean problem

To the Editors:

The rising incidence of cesarean delivery in the United States shows no signs of diminishing. In 1983 the rate was 20% while in 1965 it was 5%, equivalent to 1500 fewer cesarean births *per day*.^{1,2} The national Institutes of Health consensus development report on cesarean childbirth³ identified dystocia and repeat cesarean section as the two principal contributors to the increase. Repeat cesarean section has been studied in some detail, and several investigators have demonstrated how the repeat rate may be safely reduced.^{3,4} Dystocia has received far less attention. We wish to draw attention to a potentially confusing approach to analysis of the dystocia problem and to suggest a way to clarify the issue.

It is conventional practice to calculate the incidence of cesarean section as primary and repeat, with use of all mothers delivered, regardless of parity, as denominator. This approach disguises the influence of parity on the incidence of cesarean section for dystocia. We suggest that cesarean section rates for dystocia be calculated separately for nulliparous and multiparous women. As demonstrated in Table I, analysis of incidence of cesarean section by parity highlights its influence on the incidence of cesarean section for dystocia. The figures in the table are derived from Hermann Hospital, The University of Texas—affiliated hospital in Houston. The hospital delivers patients cared for by three different practice types: a University-based practice (30%), a Health Maintenance Organization (65%), and a group of autonomous private practitioners (5%). The overall incidence of cesarean section (19%) is almost identical to the national figure.

O'Driscoll et al.⁵ have argued that dystocia in nulliparous women is largely responsible for the current high incidence of cesarean section in the United States, an argument based on the twin assumptions that the incidence of cesarean section for dystocia is markedly influenced by parity and that cesarean delivery in a first pregnancy is likely to result in cesarean delivery in subsequent pregnancies.

Despite tacit acknowledgement of an increased incidence of cesarean section for dystocia in nulliparous women, it is unusual to see cesarean section rates analyzed by parity. Williams and Chen⁶ found a 3.5-fold increased incidence of primary cesarean birth in nulliparous women in California in the years 1978 to 1980,

Table I. Total and cesarean births at Hermann Hospital, Houston, from January 1, 1985, through June 30 1985, classified by parity and indication for cesarean birth

Indication	Parity				Total	
	Nulliparous		Multiparous			
	N	%	N	%	N	%
Dystocia	127	17.0	28	1.8	155	6.7
Repeat elective cesarean section	—		117	7.4	117	5.0
Fetal distress	37	5.0	38	2.4	75	3.2
Breech presentation	36	4.8	14	0.9	50	2.2
Other	25	3.3	27	1.7	52	2.2
Total cesarean births	225	30.1	224	14.2	449	19.3
Total births	747		1577		2324	

but the indications were not analyzed in detail. Martin et al.⁷ in their analysis of figures from a single hospital in Montreal from 1973 to 1979 demonstrated a similar trend. The National Institutes of Health consensus report,² however, did not examine the influence of parity on incidence of cesarean section for dystocia. We expect that analysis of figures from other institutions will reveal an effect of parity on the incidence of cesarean section for dystocia similar to our institution and suggest that presentation of data in this way may help focus attention on what we, in agreement with O'Driscoll et al.,⁶ suspect is the root cause of the cesarean problem: dystocia in first labors.

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Risk factors for gestational trophoblastic neoplasia

To the Editors:

We read with interest the article by Messerli et al. (AM J OBSTET GYNECOL 1985;153:294-300) concerning

risk factors associated with development of gestational trophoblastic neoplasia. The authors present evidence suggesting that gestational trophoblastic neoplasia may be part of a continuum of early (first-trimester) reproductive abnormalities. They found that women with molar pregnancy, more often reported a history of spontaneous abortion than did control subjects. We would like to stress the role that spontaneous abortions may play in the etiology of gestational trophoblastic disease, based on a study that was conducted on 96 women with previous history of the disease.¹

Of the 96 women with complete hydatidiform mole 70 were pregnant before the molar gestation. Of the 300 premolar pregnancies 72% produced living children, 16.3% terminated in spontaneous abortions, and 11.7% were artificial abortions. Following molar pregnancy these women had 69 conceptions, 71% of which terminated in delivery of a living child, 17.3% were spontaneous abortions, and 11.6% were artificial abortions (Table I).

In the evaluation of reproductive performance after occurrence of gestational trophoblastic disease, pregnancies preceding molar gestation served as controls. It is, however, conceivable that patients who eventually develop a molar pregnancy do not constitute an entirely normal group and that their reproductive history differs from that of a normal population. It would be logical to propose that the appearance of gestational trophoblastic disease represents one event in the course of pathologic reproductive performance.

To examine this thesis, the outcome of pregnancies preceding the molar gestation in 61 patients was compared with a matched group of women who never developed gestational trophoblastic disease. Patients of the gestational trophoblastic disease group and the controls were matched according to age, parity, socioeconomic status and country of origin. The reproductive performance of these two groups is shown in Table II. The incidence of both spontaneous and induced abortions was significantly higher ($p < 0.01$) in the gestational trophoblastic disease group as compared to the controls. This finding allows for some speculations as to the possible etiology of gestational trophoblastic disease. It is possible that the high incidence of spontaneous abortions in patients who eventually develop a

Table I. Outcome of pregnancy before and following molar gestation

	Delivery of a living child		Spontaneous abortion		Artificial abortion	
	n	%	n	%	n	%
Before molar pregnancy	216	72.0	49	16.3	35	11.7
After molar pregnancy	49	71.0	12	17.3	8	11.6

Table II. Outcome of pregnancies preceding gestational trophoblastic disease in 61 women compared to matched control subjects

	Live births		Spontaneous abortion		Artificial abortion		Total
	n	%	n	%	n	%	
Patients with gestational trophoblastic disease	216	72	49	16.3	35	11.7	300
Control subjects	235	84.2	26	9.3	18	6.4	279

molar pregnancy was caused by chromosomal disorders in the conceptus which in some instances led to abortion and in others to development of gestational trophoblastic disease. Since the induced abortions were all performed in the first trimester and the material was not examined for chromosomal abnormalities, it is also conceivable that among them some of the conceptuses were abnormal.

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REFERENCE

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Gossypol and lipid peroxidation

To the Editors:

The fluidity and structural integrity of plasma membranes are maintained by phospholipids. These phospholipids are rich in unsaturated fatty acids.¹ The unsaturated fatty acids of these membrane phospholipids are susceptible to lipid peroxidation. Therefore the lipid peroxide potential of a membrane and its measurement may reflect indirectly the structural integrity of membranes.² The structural integrity of membranes is important for a normal function of cells. Spermatozoal membranes are rich in phospholipids and the structural integrity of their membranes are important for their function.³ Gossypol, an effective male contraceptive apart from its reversible inhibition of sper-

matogenesis, is known to bind to spermatozoal membranes.⁴

In this report the effect of gossypol tetra-acetic acid (World Health Organization, Geneva) on lipid peroxidation of spermatozoa and erythrocytes is described to show its effect at the membrane level. Washed human spermatozoa were prepared from semen samples obtained from healthy volunteers ($n = 10$) by centrifuging the semen sample at $400 \times g$ for 20 minutes and washing it in TRIS buffer (sodium chloride, 123 mmol/L; potassium chloride, 5 mmol/L; magnesium sulfate, 1 mmol/L; TRIS, 37 mmol/L (Sigma), pH 8.0). Erythrocyte membranes are prepared with use of the procedure of Dodge et al.⁵ Spermatozoal and erythrocyte membrane suspensions were incubated in the presence of 25 μM of ferrous ions and 125 μM of ascorbate for 1 hour at 37° C. Duplicate samples of both were preincubated with gossypol (0 to 5 μM) and lipid peroxidation was induced by ferrous ions and ascorbate. The amount of malondialdehyde formed is measured by thiobarbituric acid assay. The lipid peroxide potential was measured⁶ as nanomoles of malondialdehyde $\times 10^{-8}$ spermatozoa and nanomoles of malondialdehyde per gram of hemoglobin with use of malondialdehyde dimethyl acetal as standard.

It was observed that incubation with gossypol inhibited lipid peroxidation, and the inhibition of lipid peroxidation was dose dependent (Fig 1).

Thus it appears that gossypol tetra-acetic acid binds most probably to the phospholipid-rich region of plasma membranes and prevent peroxidation of polyunsaturated fatty acids attached to these phospholipids. This action of gossypol tetra-acetic acid is not specific to spermatozoal membrane, since it is also shown to bring such inhibition with erythrocyte membranes. More details of the studies are being published elsewhere, and further studies are being conducted to elicit this action of gossypol on lipid peroxidation.

I would like to add that I am grateful to The Steering

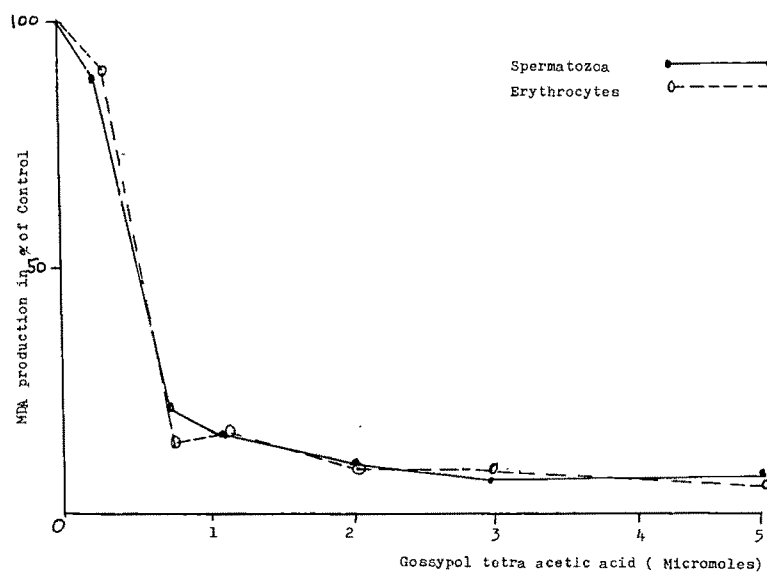


Fig. 1. The effect of gossypol on lipid peroxidation of washed human spermatozoa and erythrocyte membranes.

Committee of the Task Force on Methods for the Regulation of Male Fertility, World Health Organization, Geneva, for the supply of gossypol tetra-acetic acid.

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Books received

- The A-Z of Women's Health.** Derek Llewellyn-Jones. 250 pages, illustrated. New York, 1985, Oxford University Press. \$8.95.
- Anesthesia in Obstetrics: Maternal, Fetal and Neonatal Aspects.** Second Edition. George A. Albright, James E. Ferguson II, Thomas H. Joyce III, and David K. Stevenson. 622 pages, illustrated. Stoneham, Massachusetts, 1986, Butterworth Publishers. \$69.95.
- Baby and Child A to Z Medical Handbook.** Dr. Miriam Stoppard. 320 pages, illustrated. Tucson, 1986, The Body Press. \$19.95.
- Blood Disorders in Pregnancy.** Russell K. Laros, Jr. 245 pages, illustrated. Philadelphia, 1986, Lea & Febiger. \$32.50.
- Cancer in Pregnancy. Therapeutic Guidelines.** Edited by H. Hugh Allen and Jeffrey A. Nisker. 291 pages, illustrated. Mount Kisco, New York, 1986, Futura Publishing Company. \$32.50.
- The Health of Adolescents and Youths in the Americas.** Pan American Health Organization. 327 pages, illustrated. Washington, D. C., 1985, Pan American Health Organization. \$10.00.
- Mother and Child Health. Delivering the Services.** Second Edition. Cicely D. Williams, Naomi Baumslag, and Derrick B. Jelliffe. 316 pages, illustrated. New York, 1986, Oxford University Press. \$24.95.
- Over the Hill But Not Out to Lunch.** Lloyd Kahn, Jr. 146 pages, illustrated. Bolinas, California, 1986, Shelter Publications Inc. \$8.95 (soft cover).
- Oxford Reviews of Reproductive Biology.** Volume 7. Edited by J. R. Clarke. 409 pages, illustrated. New York, 1985, Oxford University Press.
- Planning for a Healthy Baby.** A Guide to Genetic and Environmental Risks. Richard M. Goodman. 256 pages. New York, 1986, Oxford University Press. \$16.95.
- Retained Common Duct Stones.** Edited by Roger W. Motson. 150 pages, illustrated. Orlando, Florida, 1985, Grune & Stratton, Inc. \$38.50.
- Surgery of the Breast. Diagnosis and Treatment of Breast Diseases.** Edited by J. O. Strombeck and F. E. Rosato. 336 pages, illustrated. New York, 1986, Thieme, Inc. \$98.00.
- Textbook of Laparoscopy.** Jaroslav F. Hulka. 152 pages, illustrated. Orlando, Florida, 1985, Grune & Stratton, Inc. \$49.50.
- With Child: One Couple's Journey to Their Adopted Children.** Susan T. Viguers. 226 pages. New York, 1986, Harcourt Brace Jovanovich, Publishers. \$15.95.

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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1986 Update of Controversies in the Management of Gynecologic Malignancies, September 20, 1986, Hilleboe Auditorium, Roswell Park Memorial Institute, Buffalo, New York. For further information contact: M. Steven Piver, M.D., Roswell Park Memorial Institute, 666 Elm St., Buffalo, NY 14263. Tel.: (716) 845-3110.

Eighteenth Memphis Conference on the Mother, Fetus and Newborn, September 25-26, 1986, The Peabody Hotel, Memphis, Tennessee. Sponsored by The University of Tennessee College of Medicine. For further information contact: Jean Bryan, Office of Continuing Medical Education, 800 Madison Ave., Memphis, TN 38163. Tel.: (901) 528-5547.

Birth Defects Symposium XVII: Nucleic Acid Probes in Diagnosis of Human Genetic Diseases, Albany Hilton Hotel, Albany, New York, September 29-30, 1986. Sponsored by New York State Health Department, Wadsworth Center for Labs and Research. For further information contact: Ann M. Willey, Ph.D., Wadsworth Center for Labs and Research, Rockefeller Empire State Plaza, Albany, NY 12201.

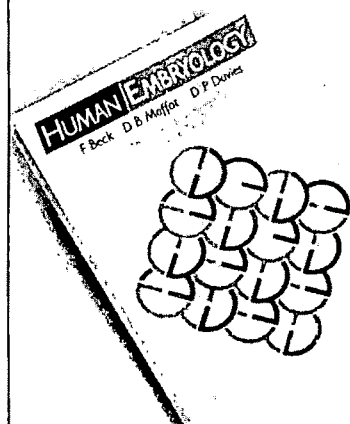
12th Annual Obstetrics and Gynecology Review Course & Pathology Course. The University of Miami School of Medicine will hold its 12th

Annual Obstetrics and Gynecology Review Course, October 12-20, 1986, and Pathology Course, October 21-24, 1986. The courses will be held at the Sheraton Royal Biscayne Hotel, Key Biscayne, Florida. For further information contact: Patti Mundy, Department of Obstetrics and Gynecology, University of Miami School of Medicine, P. O. Box 016960 (R-115), Miami, FL 33101. Tel.: (305) 549-6944.

Perinatal Medicine: 1986—Back to Basics, October 16-17, 1986, Northwestern University Medical School, Chicago, Illinois. For information contact: Paula Punttenney, Director, Alumni Center for Continuing Education, Northwestern University Medical School, 301 E. Chicago Ave., Chicago, IL 60611. Tel.: (312) 908-8533.

National Abortion Federation wishes to announce its Fall Risk Management Seminar, "Pain and Pain Management," October 26-27, 1986, Omni Georgetown at P Street, Washington, D. C. For more information, please contact NAF: (202) 546-9060.

Hysteroscopy Workshop—Panoramic-Contact-Microcolpohysteroscopy: Didactic and Practical Hands-on Experience, November 14-15, 1986, Northwestern University Medical School, Alumni Center for Continuing Education, Chicago, Illinois. For information contact: Paula Punttenney, Alumni Center for Continuing Education, Northwestern University Medical School, 301 East Chicago Ave., Chicago, IL 60611.



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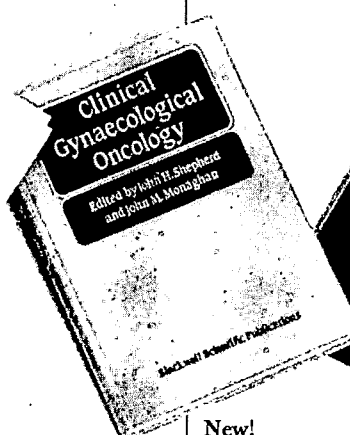
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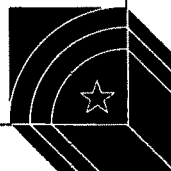
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Address all inquiries to Paul G. McDonough, M.D., Department of Obstetrics and Gynecology, Reproductive Endocrine Section, Medical College of Georgia, Augusta, GA 30912.

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Department of Obstetrics and Gynecology
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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

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CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS: An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group in terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smokers) are at a major predisposing condition to myocardial infarction are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 4.5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified. It is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** An analysis of data derived from several national adverse reaction reporting systems, British investigators conclude that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor for this finding. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000); the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until large numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time and from a previously reported prospective study of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth; with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if this is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. **CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. **HEPATIC TUMORS:** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral



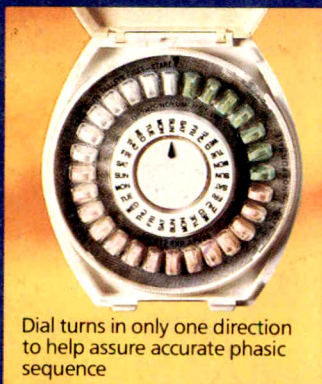
Enhanced patient compliance

contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives and natural estrogens) during pregnancy or attempted treatment for threatened abortion. Some of these exposures were very short and involved only one menstrual period. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than that in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used, and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of oral contraceptive users. Women who have had hypertension during pregnancy should be carefully observed during use of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBOHYDRATE AND LIPID METABOLIC EFFECTS:** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE:** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. **BLEEDING IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to another contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolism. Women with a past history of amenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 9. **ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **BREAST FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progesterone preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause an increase in the incidence of venous thromboembolism, and only with careful monitoring in patients with degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised that oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention b. Increased prothrombin and factors VII, VIII, IX, and X c. decreased antithrombin 3. increased norepinephrine-induced platelet aggregability c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased prehepatic uptake e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenyltolonamide, ampicillin, griseofulvin, and chloramphenicol. **LABORATORY TESTS:** **PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS AND WARNINGS.** **ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS). Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms, such as abdominal cramps and bloating. Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.



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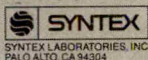
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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.



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WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. 2. Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. 3. Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. 4. Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. 5. Usage in pregnancy is not recommended (see Warning box). 6. Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influ-

enced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: **Pregnancy:** (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T₃ uptake values).

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TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.



American Journal of Obstetrics and Gynecology

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September

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Miles Pharmaceuticals Announces...

New Mycelex[®] Twin Pack (clotrimazole)

One-day therapy

Mycelex[®]-G 500mg
(clotrimazole) Vaginal Tablet

**The shortest regimen
for vulvovaginal
candidiasis**

In the case of severe vulvovaginitis
due to candidiasis, longer antimycotic
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of prescribing information.

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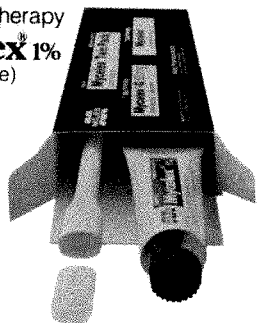
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Indications

MYCELEX-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one-day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.

MYCELEX CREAM is indicated for the topical treatment of dermal infections due to *Candida albicans*.

Contraindications

MYCELEX-G 500 mg Vaginal Tablets and MYCELEX CREAM are contraindicated in women who have shown hypersensitivity to any of their components.

Warnings

MYCELEX-G 500 mg Vaginal Tablets: none. MYCELEX CREAM is not for ophthalmic use.

Precautions

MYCELEX-G 500 mg Vaginal Tablets: If there is a lack of response to the Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

MYCELEX CREAM: If irritation or sensitivity develops with the use of Mycelex Cream, treatment should be discontinued and appropriate therapy instituted.

Carcinogenesis

MYCELEX-G 500 mg Vaginal Tablets: No long-term studies in animals have been performed to evaluate the carcinogenic potential of 500 mg Vaginal Tablets intravaginally. A long-term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

Usage in Pregnancy: Pregnancy Category B:

MYCELEX-G 500 mg Vaginal Tablets: The disposition of ¹⁴C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy. MYCELEX CREAM: In the first trimester of pregnancy, Mycelex Cream should be used only when considered essential to the welfare of the patient.

Adverse Reactions

MYCELEX-G 500 mg Vaginal Tablets: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

MYCELEX CREAM: The following reactions have been reported in connection with the use of this cream: erythema, stinging, blistering, peeling, edema, pruritus, urticaria, and general irritation of the skin.

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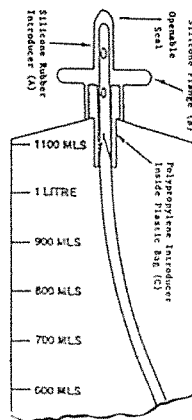
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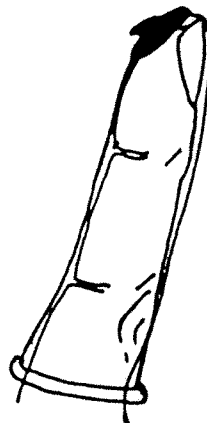
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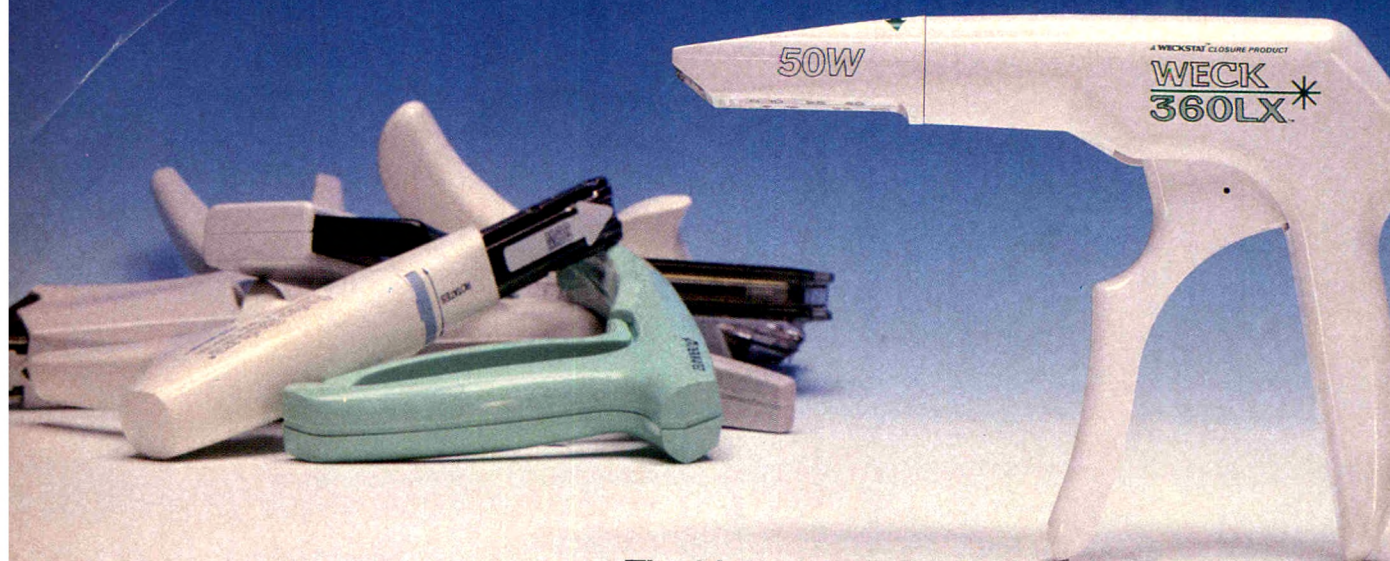
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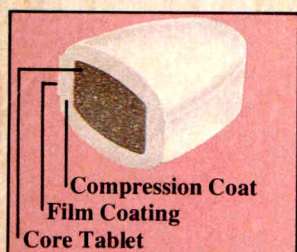
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Vitamin E, IU
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Vitamin B₁₂, mcg
Biotin, mg
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400	100
30	100
90	150
1	125
2.55	150
3	150
20	100
10	400
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15	150
200	15
150	100
60	333
100	22
2	100
15	100

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none

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*In non-pregnant patients, 3-day treatment is recommended. Treatment may be extended an additional 3 days, if necessary.

1. Droegemueller W, Adamson DG, Brown D et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 64:530-534, 1984.

Please see brief summary of prescribing information on next page.

The Only 3-Day Cream*

Think vaginal cream 2.0% Femstat® (butoconazole nitrate) The Only 3-Day Cream

Brief Summary

INDICATIONS:

FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by *Candida* species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:

FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:

General:

If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient:

Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis:

Animal studies have not been done.

Mutagenesis:

Mutagenicity studies were negative.

Impairment of Fertility:

Animal studies showed no impairment of fertility.

Pregnancy Category C:

Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:

Use with caution.

Pediatric Use:

Safety and efficacy have not been established.

ADVERSE REACTIONS:

Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:

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December 1985



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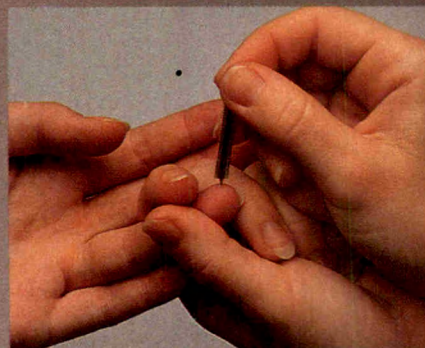
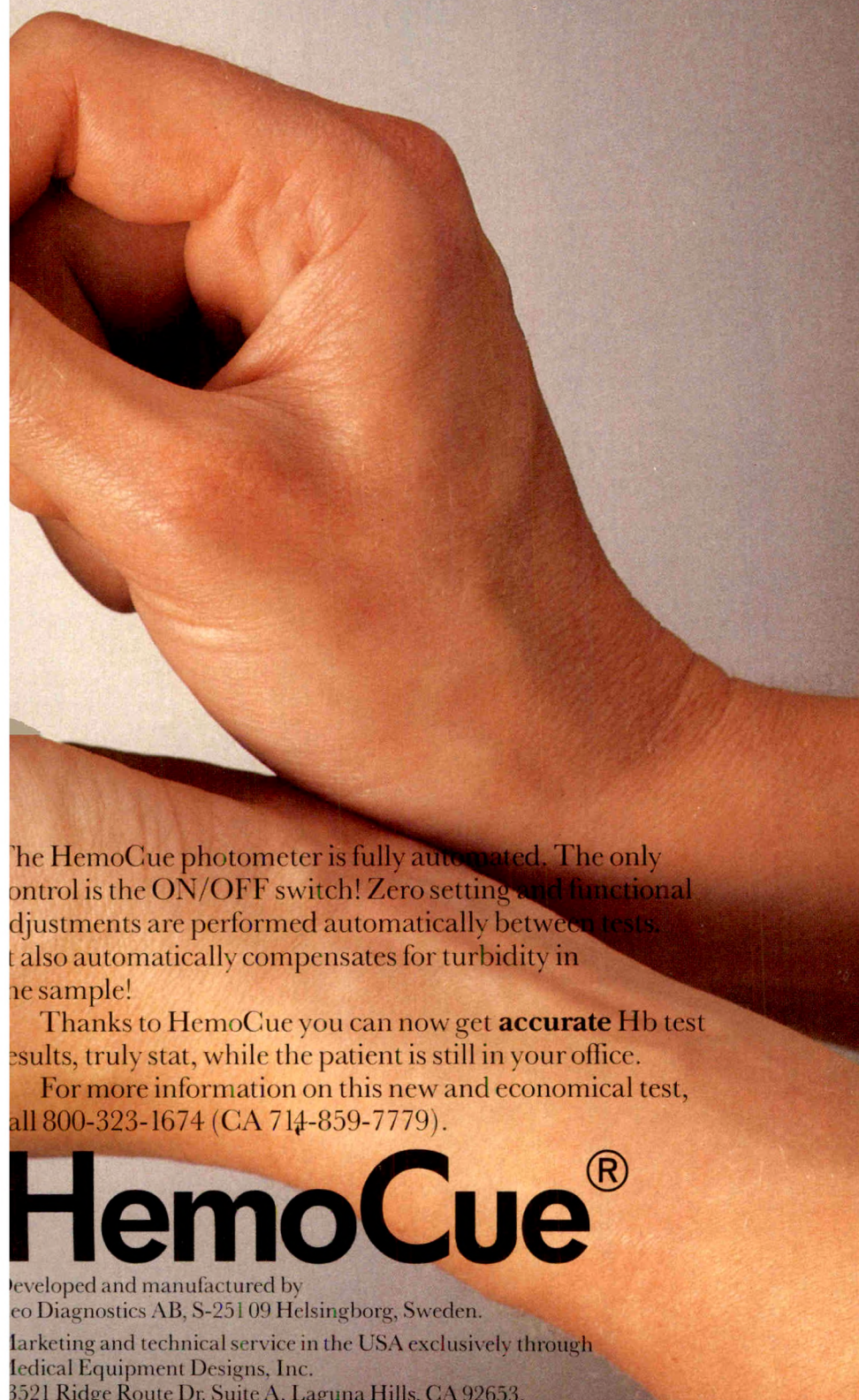
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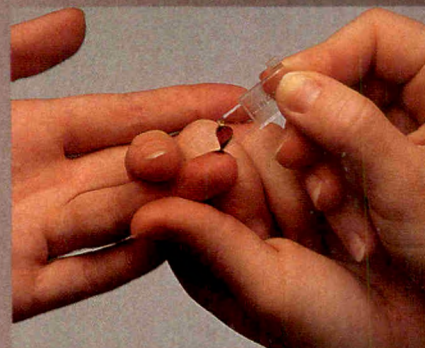
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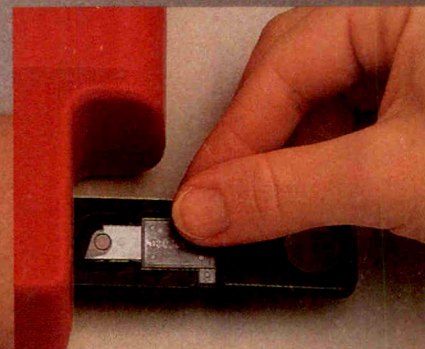
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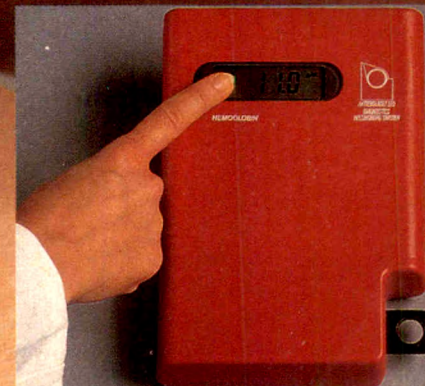
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* Due to susceptible strains of indicated organisms.

† Dosage in renally compromised patients may require adjustment. Please see complete prescribing information.

‡ *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Harding G.K.M., et al.: Rev. Infect. Dis. 6(Suppl. 1) S283-S292, 1984.

2. Fu, K.P., and Neu, H.C.: Antimicrob. Agents Chemother. 17:583-590, 1980.

3. Aldridge, K.E., et al.: Antimicrob. Agents Chemother. 26:410-413, 1984.

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URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli*; *Pseudomonas* sp. including *Ps. aeruginosa*; *Proteus mirabilis*; *P. vulgaris*; *Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens*; *Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae*.

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli*; *Staphylococcus epidermidis*; *Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis*; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli*; *Bacteroides* sp. including *B. fragilis*; *Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis*; *Escherichia coli*; *Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis*; *Serratia* sp.; *Enterobacter* sp.; including *B. fragilis*; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis*; *Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae*. 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae*.

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use. Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox', nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatinine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the most frequent adverse reactions (*greater than 1%*, but less than 5%) are: **Hypersensitivity**—Rash, pruritus, fever. **Hepatic**—Transient elevation in SGOT, SGPT and alkaline phosphatase. **Hematologic**—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. **Local**—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (less than 1%) are: **Hypersensitivity**—Numbness has been reported rarely. **Hepatic**—Elevation of bilirubin has been reported rarely. **Renal**—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. **Hematologic**—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. **Genitourinary**—Vaginitis has rarely occurred. **Gastrointestinal**—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

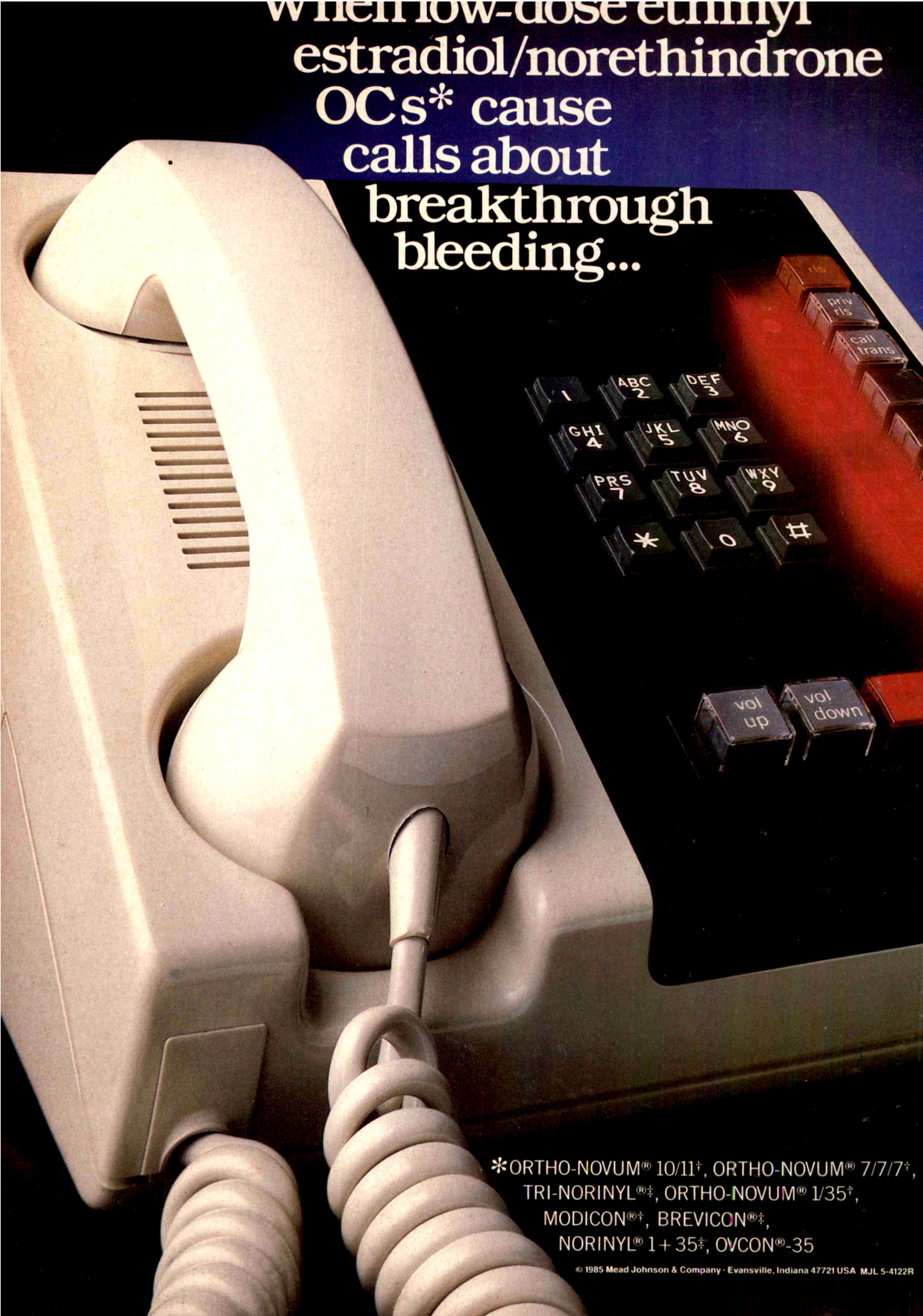
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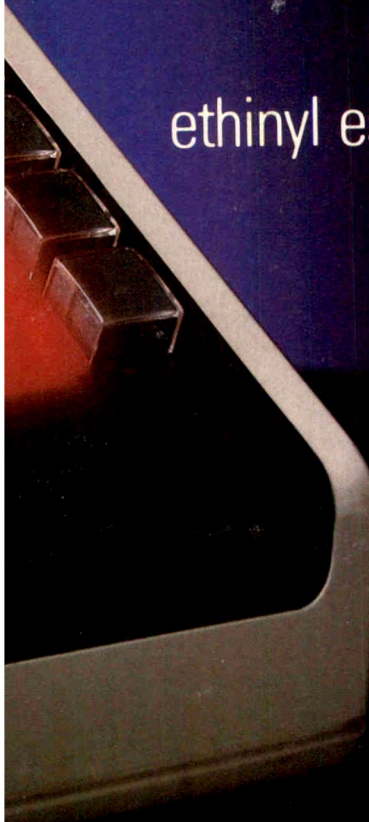
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when low-dose ethinyl
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OCs* cause
calls about
breakthrough
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*ORTHO-NOVUM® 10/11†, ORTHO-NOVUM® 7/7/7‡,
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MODICON®†, BREVICON®‡,
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The only 50-mcg OC containing ethinyl estradiol & norethindrone

eliminates the "estrogen variable" by providing
the same hormones as her low-dose OC

- ethinyl estradiol, the preferred estrogen—not mestranol, an estrogen that shows highly variable metabolism among individuals¹
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1. Mishell DR Jr: Oral steroids, in Mishell DR Jr, Davajan V(eds): *Reproductive Endocrinology, Infertility and Contraception*. Philadelphia, FA Davis Co, 1979, p 489.

†Registered trademark of Ortho Pharmaceutical Corp. for norethindrone and ethinyl estradiol tablets.

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For Brief Summary for OVCON-50 and OVCON-35, please see following page.

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OVCON®-50
(norethindrone [1 mg] and
ethinyl estradiol [0.05 mg]) tablets

OVCON®-35
(norethindrone [0.4 mg] and
ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg. or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2 to 3 times greater than nonusers and the risk of thrombotic stroke was 4 to 5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/taxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism, including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000, ages 35-44, 33/100,000, ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/taxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use. The risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of females exposed to oral progestogens and/or estrogens during pregnancy. It has been shown that females exposed to utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual

abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gall Bladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) **Ectopic Pregnancy.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breast Feeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised if oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalene retention; b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin; c. increased norepinephrine-induced platelet aggregability; d. Increased thyroxine binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T₄ by column, or T₄ by radioimmunoassay. Free T₄ resin uptake is decreased, reflecting the elevated TBG. Free T₄ concentration is unaltered; d. Decreased preheparin-induced platelet aggregation; e. Reduced response to metoprolol test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, catarracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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TRANSACTIONS OF THE FORTY-EIGHTH ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Women in transition

Presidential address

Joseph B. McCoy, M.D.

Charlotte, North Carolina

In 1938, the founding fellows of this association came together to establish a new institution; an institution of study, of teaching, and of collegial discourse to improve the practice of obstetrics and gynecology. To these founders we acknowledge our appreciation for their part in the advances in medicine we see today and from them we accept the responsibilities that we shall carry on in the future.

From the mountaintop of five decades we look down on advances in medical care that only the clairvoyant could have foreseen or predicted. Infant mortality has decreased from 47 per 1000 live births in 1940 to 11.9 per 1000 live births in 1981. Simultaneously maternal mortality has dropped from 37.5 per 100,000 live births in 1940 to 8.5 per 100,000 live births in 1981.¹ Other changes that have saved lives are the widespread use of the Papanicolaou smear, the virtual conquering of infectious diseases by antibiotics, and the prevention of hemorrhagic death by the availability of blood banks. These routinely accepted methods of treatment were not available to our founding fellows. Additionally, the rapid expanse of medical knowledge in recent years has given us the ability to control Rh sensitization, to essentially eradicate rubella, and to cure many of the sexually transmitted diseases, some of which were not even known when this organization was founded. Advances in knowledge and in technology have made possible in vitro fertilization, intrauterine surgical procedures, ultrasound, endoscopy, laser surgical procedures, and gene splicing.

This catalogue lists only the advances in medical science that have affected the practice of our profession. It is not enough to marvel at them. It is not enough to settle back in comfortable satisfaction and proclaim what we have done. Beyond them are profound changes in life-style, in economic consequences, in political alignment, in family structure, and in the very lives of the patients whom we serve. The woman is the consumer of our services. How has she changed?

First to be considered is the economic sector. Women in dramatic numbers have entered the work force. In 1975 39% of all women worked. This increased to 51% in 1984.² In the professions—medicine, law and theology—and in the business world the number of women is increasing rapidly. In the past women have not been expected to have a career or acquire great wealth or even support themselves. What they have been expected to do is to marry, have children, and care for the family. Now women are entering the work force either because they choose to or because they must work to support themselves or to supplement income for their families. While more women are working, figures from the Bureau of Census belie the conclusion that women in general are improving their position in the economic world. Women earn considerably less than men. In 1955 the full-time working woman earned 64¢ for every dollar a man made. In 1983 the figure was the same—64¢ versus \$1.³ The wage gap has not changed in three decades. A major reason for this disparity is that women are concentrated in twenty of the 420 occupations listed by the United States Department of Labor—mainly jobs in retail sales, clerical work, low-skill manual labor, and services that typically have low wages and are dead-ended.⁴ Women's work has historically been characterized by its low pay, interruptability,

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monotony, and repetition. One article reports that 46% of all working women can be found in four occupations: clerks, saleswomen, waitresses, and hairdressers.⁵

Even more discouraging is the growing number of women whose income falls below the poverty line—a direct result of the breakup of marriages and the rapid rise in the number of single-parent families. A 1984 Census Bureau statistic indicated that single-parent households represent 26% of all families with children under 18 years of age and that 90% of these households are headed by women.⁶ Economic hardships that these women encounter while trying to raise a family alone have led to a new trend that social scientists have labeled “the feminization of poverty.” Another large segment of poverty includes the female head of household over 65 years of age. Sadly, the number of women and children in poverty has increased in recent times. Without assistance, hundreds of thousands of women in our country can expect to live out a grim scenario. They can expect to grow up as a member of a poor family, secure a job that will keep them a member of the working poor, raise children alone in poverty, and finally join the majority of the population of female aged poor. On the positive side, many economists predict that the overall earning power of women will increase. They attribute this to the approaching seniority of the wave of women who came into the work force in the 1970s.⁷ Hopefully, with more training and higher levels of education and with attitudinal changes of the public, the gap will narrow significantly in the next decade.

On the political front women have had some headline successes: mayors of major cities, United States senators and representatives in congress, cabinet officers, and even a candidacy for vice-president on a national ticket. In Great Britain, Israel, and India the government itself has been headed by women. With women making up 53% of the voting age population and the number of women elected to state legislatures more than tripling in the past fifteen years, there is reason to look for further participation in the political process.⁷ Polls indicate that women are more likely to support funds for social progress, peace initiatives, and programs with emphasis on education, environment, and health.⁷ With their enormous voting power women have the power to make things happen if they choose to use it.

Nowhere has the change in women's status been more pronounced than in the institution of the family. In the 1980s, according to the Census Bureau, in more than 60% of families both husband and wife work and only 12% have a working father, a full-time homemaking mother, and a number of children. Sixty-six percent of women as single parents are in the work force.⁷ The traditional family is in a state of flux. The personal dilemma of the woman of the 1980s is how to balance demands of a career with the responsibility of being a

wife and mother. As a result, marriage roles have changed. Women are getting married later and having fewer children. For many working mothers the myth of superwoman has given way to the reality that working in the marketplace and raising children is a difficult and sometimes overpowering task. Family structure itself has changed dramatically with one divorce in six marriages in 1940 and one divorce in two marriages in 1980. Three fourths of all divorced persons remarry, which suggests that the family as an institution is not being rejected.⁸ The traditional family unit no longer exemplifies two-parent families, lifetime marital commitment, marriage with children, and a family circle that extends from young adult to postretirement stages. Alternatives to the traditional family style are growing and becoming more diversified and I expect that every family here is in some way experiencing these changes.

The fourth category for consideration is women's health. Probably the most important change of all is women's increased ability to control their reproductive lives with the advent of “the pill,” along with improvements in other forms of contraception. This has enabled our patients to have a planned family with the average number of children being 3.6 in 1960 and 1.8 in 1984.⁹ The pill had a stunning effect on American society. Beginning in the 1960s women could experience sex without fear of pregnancy and, for the first time, know the kind of sexual freedom that only men had known. From our vantage point in the 1980s it is difficult to imagine the beginning of the struggle to change laws and attitudes about contraception. As late as 1960, a couple using contraceptives in their own Connecticut bedroom was committing a felony. The last birth control laws were in effect in Massachusetts until 1972. The sexual revolution is not without a price. There is no social change that comes without new conflicts and emotional conflicts have been the most widespread side effect of the pill.⁹ One woman's freedom may be another woman's license and another woman's insecurity. Also impacting women's health was the supreme court's ruling in 1973 making abortion legal. This decision, though controversial, did take abortion out of the back alleys and into a safe and sterile environment. Women are still in sexual transition.

Perhaps a symptom of women's and society's confusion over female sexuality is the high number of illegitimate births. Teenage pregnancy in this country is a growing health and sociologic problem. The Alan Guttmacher Institute reported that the rate of illegitimate teenage pregnancy in the United States was more than twice the rate of industrial European countries though sexual activity is said to be the same.¹⁰ This problem has complex cultural roots but sex education and the availability of contraceptives to this large segment of females will be a start toward a solution. Dr.

Luella Klein, immediate past President of the American College of Obstetricians and Gynecologists, has been particularly effective in bringing this to the attention of physicians and the public in general.

A key factor in the focus on women's health is the dramatic increase in the number of female physicians. One third of medical students today are women. Dr. Keith Russell predicts that in the very near future our specialty will be equally divided between men and women.⁷

This focus on women's health has been significantly advanced by the insistence by women of better health care. Many of you remember that a typical labor in the 1940s was managed with "twilight sleep," a combination of morphine and scopolamine, and delivery was accomplished with drop ether or chloroform for anesthesia. Fortunately, this has been replaced, in part because of the demand of patients for alternatives such as the various programs on childbirth preparation, less depressive forms of anesthesia, and, more important, the cooperation of laboring women and support from their husbands. Little headway has been made in decreasing the mortality of breast cancer but alternative methods of therapy have made treatment more acceptable to some women. Unfortunately, lung cancer and chronic respiratory diseases have hung heavy over the smoking woman and more public awareness of our environmental danger may reverse this trend. Being female continues to offer some protection from heart disease though with an aging female population this area must be considered a high priority for further research.

One outstanding statistic in women's health care is that in a span of 32 years, from 1950 to 1982, the life expectancy of a female born in the United States increased from 71.1 to 78.2 years—more than 7 years of longevity.⁸ That this increase in longevity is due to medical science is only part of the answer. Improved nutrition, education, and environment have certainly been factors. Then that certain indefinable biologic advantage that has always made women live longer than men must be given credit.

All things considered, the female in the United States is healthier than she was in earlier years. An article in the *Ladies Home Journal* sums it up this way: "Women in the '80's are better educated, healthier, and live longer, fuller lives although their earning power is yet to reach that of men. Women have also stretched their adult years by postponing marriage and motherhood until they get an education and start a career. They have managed to combine work outside the home with family life; and as women have changed, society has had to respond. Day care centers have sprouted in suburbs and towns. Supermarkets are open 24 hours a day. Repairmen make evening calls. Best of all, husbands are helping out."⁹

Socially, economically, and physically women's options are expanding. A major factor in this expansion of opportunity is women's increasing willingness to vocalize their needs and wants. In 1969 in Boston a small group of women gathered to discuss women and their bodies. Out of that beginning grew The Boston Women's Health Collective. They have published a book by and for women now widely known as *Our Bodies Ourselves*. This is a quote from a paragraph in the preface of the book: "In the beginning we called ourselves the doctors' group. We had all experienced similar feelings of frustration and anger towards specific doctors and the medical maze in general and initially we wanted to do something about these doctors who were condescending, paternalistic, judgmental, and noninformative."¹⁰ If only a small percentage of our patients feel this way about their physicians then we must look seriously at ourselves and the way we dispense our medical services. Each of us can be proud of the advances made in the health care of women during our personal careers but this is not enough. It no longer suffices to be a good technical surgeon or dispenser of the healing herbs or attendant at the birthing of the firstborn. We must concern ourselves with looking on our patients not as a disease entity or as a neurotic housewife, but as a whole person, one who has feelings and a thinking mind, one who is sensitive to her own wants and needs, and these wants and needs will not be the same with every patient. Many women, both as consumers and as health workers, are making a radical challenge to the medical care system as we have known it. We, as physicians, must respond to this challenge and be a part of a medical care system that will benefit all women.

In spite of the many changes in women's lives over the years, motherhood remains both an essential and a cherished role. All humanity reveres a mother. She nurtures and loves our children. She offers them succor when they are ill, frightened, or in pain. She comforts their father in times of stress and hardship. All of this and more does a mother give—but this mother represents many women who are frightened, who are poor, who are insecure, who have pain, who feel neglected, unwanted, and unloved. She is our patient also.

Ours is the only medical specialty that treats women exclusively. We must be her champion. We must listen to her needs and help provide for them. No longer can we be content to treat a disease and neglect to listen to our patient when she is crying out to be recognized as a human being. We must be women's advocates in health matters—yes, but more than that, we must be women's advocates in all that affects her, whether it be in the marketplace, in her political life, or in the family.

We must support her as she seeks equality in the work force and fairness in pension systems and social security. We must support her when she seeks to be heard

in the political arena. We must be supportive as she attempts to balance the multineeds of her family. Perhaps our country's greatest wealth is her women, and these women want and deserve to be heard. They are our patients. •

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Intravenous versus intraperitoneal administration of dextran in the rabbit: Effects on fibrinolysis

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Adhesions are the leading cause of small bowel obstruction and a frequent cause of failure of infertility operations. Fibrinolysis is involved in the formation and resolution of adhesions. Although intravenous dextran (Macrodex) is known to augment intravascular fibrinolysis, the effects of intraperitoneal dextran (Hyskon) on fibrinolysis have not been extensively studied. A fibrin plate assay system was used to assess plasminogen activator activity of rabbit peritoneum and plasma after treatment with intraperitoneal or intravenous dextran 70. Hyskon significantly reduced the ability of severe trauma to depress plasminogen activator activity of visceral peritoneum and was capable of direct plasminogen activation. Untraumatized or minimally traumatized peritoneum was not affected, nor was plasminogen activator activity of plasma. Pulmonary effusions and perioperative deaths were significantly associated with the use of Hyskon. (AM J OBSTET GYNECOL 1986;155:464-70.)

Key words: Dextran 70, plasminogen activator activity, fibrinolysis, pulmonary effusion

Postoperative intraperitoneal adhesions are the leading cause of small bowel obstruction as well as the most frequent cause of failure of infertility operations. The

cause and prevention of adhesions is the subject of several recent comprehensive reviews.¹⁻³ The prevention of this potentially life-threatening complication is still incompletely understood. Recently, however, it has been shown in humans that the use of intraperitoneal 32% (w/v) 70,000 molecular weight dextran in 10% dextrose (Hyskon, Pharmacia) is an effective adjunct for the prevention of postsurgical adhesions.⁴

Why dextran is effective is not clear. Permanent adhesions persist when the early fibrinous adhesions that universally form between injured and adjacent tissues are not lysed, as a result of failure to activate tissue plasminogen activator.⁵⁻⁷ Hyskon is a very viscous, hypertonic solution that coats tissues and by its "siliconizing" effect may prevent adherence of injured surfaces. In addition, it produces a temporary ascites,

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which may prevent approximation of tissues. Both the "siliconizing" and "hydroflotation" theories imply that early adhesions do not form and therefore cannot persist.

Studies in dogs and rats have shown that severe injury (for instance, ischemia, crushing, abrasion) causes a depression of tissue fibrinolysis mediated by inhibitors of plasminogen activator. This correlates with the formation of permanent fibrous adhesions.⁵⁻⁷ Intravenous dextran augments fibrinolysis in several ways, including depression of plasminogen activator inhibitors.⁸ The possibility exists that intraperitoneal dextran may act directly on the peritoneum by the same pathway, thereby enhancing the lysis of early fibrinous adhesions. In spite of the important role of intact fibrinolysis in the resolution of postoperative adhesions, the effects of intraperitoneal dextran on tissue fibrinolysis have not been studied. It is also not clear whether intraperitoneal dextran affects intravascular fibrinolysis, although bleeding problems have not been encountered with its use.⁴

With the use of a modification of the Astrup fibrin plate assay of plasminogen activator,⁹ the following experiments were performed to determine if either intraperitoneal dextran or intravenous dextran affected plasminogen activator activity of traumatized or untraumatized peritoneum. Variations of this methodology were used to establish the role of trauma-mediated depression of plasminogen activator in the etiology of permanent adhesions.^{6,7} An attempt was also made to detect effects of either intraperitoneal or intravenous dextran on intravascular plasminogen activator activity.

Material and methods

Adult New Zealand White rabbits of either sex weighing between 3.5 and 5.5 kg were used in this study. The animals were housed in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care and were fed and cared for according to National Institutes of Health guidelines. The experimental protocol was approved by the Laboratory Animal Medicine Ethics Committee of The University of South Florida College of Medicine.

In experiment 1, they were randomly assigned to control group A receiving 10 ml/kg of intraperitoneal 10% dextrose (two male and two female animals) or experimental group C receiving 10 ml/kg of intraperitoneal 32% dextran 70 in 10% dextrose (Hyskon, Pharmacia) (one female and five male animals). We originally intended to work with large female rabbits, but after the experiment was underway, only male rabbits of suitable size were available. In experiment 2, 15 male rabbits were randomly assigned to one of three treatment groups. Group A (four rabbits) received 10 ml/kg of intraperitoneal 10% dextrose (controls).

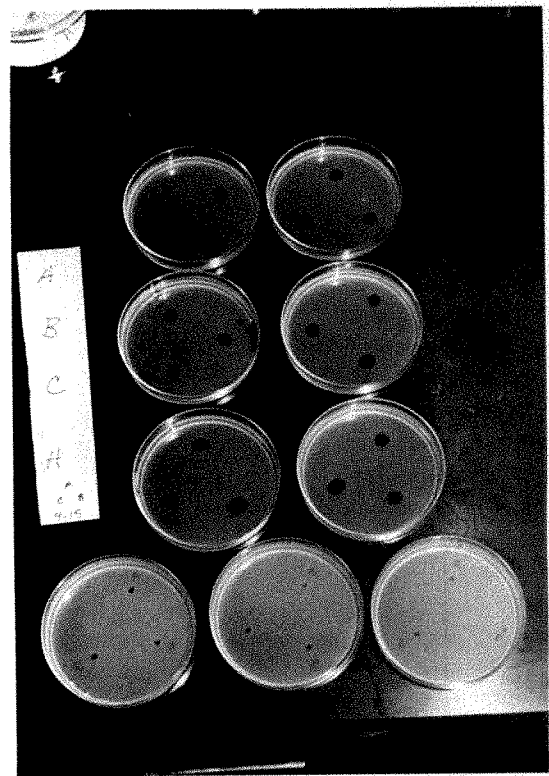


Fig. 1. Zones of lysis produced by 20 λ drops containing blood euglobulin fraction after incubation at 37° C for 18 hours. Bottom row plates were heated for 15 minutes at 80° C before samples were placed on them.

Group B (five rabbits) received 7.5 ml/kg of intravenous 6% dextran 70 in normal saline solution (Macrodex, Pharmacia) and 5 ml/kg of intraperitoneal 10% dextrose. Group C (six rabbits) received 10 ml/kg of intraperitoneal Hyskon. Hyskon and Macrodex were commercially available sterile preparations. Ten percent dextrose solution was prepared in the laboratory and immediately passed through a Nalgene Filter (0.20 μ m) to provide a sterile preparation.

After preanesthesia with intramuscular ketamine (20 mg/kg), experimental solutions were instilled via small laparotomy incisions with animals under surgical anesthesia (2.5% to 5.0% halothane in oxygen). Incisions were closed in two layers with the use of 2-0 vicryl and 3-0 silk sutures. Macrodex was infused via an ear vein during a 10- to 15-minute period. Food was withheld 18 hours before operation, but water was given as desired.

Fibrinolytic activity of the euglobulin fraction of plasma obtained before and 48 hours after treatment and of peritoneum (visceral and parietal) harvested 48 hours after treatment was assayed by measuring the lytic zones produced after samples were incubated on fibrin plates⁹ (Figs. 1 and 2).

Plates were prepared by adding 10 units of thrombin

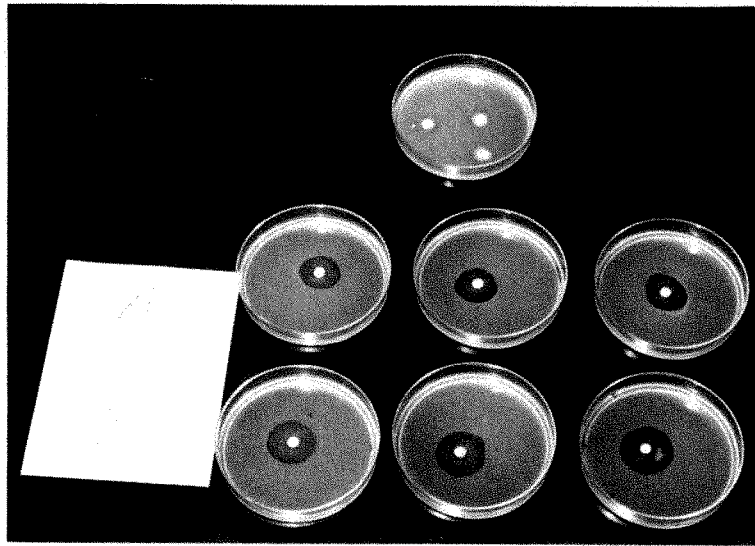


Fig. 2. Zones of lysis produced by cecal biopsy specimens after incubation on fibrin plates for 18 hours at 37° C. The top plate was heated for 15 minutes at 80° C before samples were placed on it.

(Thrombostat, Parke-Davis) to a tube containing 4.5 ml of 500 mg/100 ml bovine fibrinogen (Sigma F-8630) in a standard sodium barbital buffer (pH 7.35) and 4.5 ml of 0.05 mol/L calcium chloride. After one rapid inversion, the mixture was poured into a sterile 100 by 150 mm Petri dish and allowed to clot, producing a fibrin layer of uniform thickness. Samples were also incubated on plates that had been heated for 15 minutes at 80° C. Since plasminogen is heat labile, activity present only on unheated plates was due to plasminogen activator and not plasmin or other proteolytic enzymes, for example, trypsin. In order to minimize variation between plates, all plates for each experiment were prepared at the same time from the same batch of fibrinogen solution. Drops containing known amounts of urokinase (Sigma U-8627) were also incubated on random plates from each batch to check for variation.

Blood samples obtained by cardiac puncture were drawn directly into precooled vacuum tubes containing 0.5 ml of citrate. After centrifugation in a refrigerated (4° C) centrifuge at 2500 rpm for 20 minutes, the euglobulin fraction of the plasma (1 ml diluted in 15 ml of cold water) was precipitated by acidification with 1% acetic acid to pH 5.8. After a waiting period of 30 minutes at 4° C, the sediment was isolated by centrifugation (4° C, 3000 rpm, 10 minutes). The precipitate, containing the fibrinolytic fraction without the fibrinolytic inhibitors present in whole plasma, was then reconstituted in 1 ml of buffer. The euglobulin fraction drops (20 λ) were incubated in triplicate on heated and unheated fibrin plates for 18 hours at 37° C allowing a semiquantitative comparison of intravascular fibrinolytic activity.

After 48 hours and with animals under adequate surgical anesthesia, peritoneal samples were harvested. Both visceral and parietal peritoneal biopsies were performed, and an attempt was made to determine whether treatment affected the trauma-mediated decrease in fibrinolysis observed by other investigators.⁸⁻¹¹ The cecum was isolated and cross clamped about 5 cm from the distal end. The proximal segment was then abraded 25 times with a 4 by 4 gauze sponge. In experiment 1, samples of cecum were gathered with a 6 mm dermal punch. In an attempt to be frugal, which proved to significantly affect the outcome, dermal punches were used until they were too dull to be effective. After it became apparent that this method was providing unwanted trauma, the experimental protocol was changed. In experiment 2, the cecum was clamped and abraded, then excised and opened along its mesenteric border; it was rinsed thoroughly in buffer, then placed serosal side up in a shallow glass dish containing buffer. Sharp dermal punches (discarded after three uses) were used to obtain samples, which were then placed serosal side down on heated and unheated fibrin plates. Lytic zones were measured after incubation at 37° C for 18 hours. Biopsy specimens bearing parietal peritoneum were gathered from the abdominal wall on either side of the midline incision. One side was sponge abraded before excision. After tissue harvest was complete and while still anesthetized, animals were put to death by exsanguination. Ascites and pleural fluid were quantified when present. Data from the two experiments were pooled when appropriate and were analyzed by Student's *t* test or χ^2 . A $p < 0.05$ was considered to be significant.

All solutions including buffer were also incubated on

Table I. Average diameters of lytic zones produced by blood euglobulin fraction drops before and 48 hours after treatment

Group	Experiment 1: Alert (mm)		Experiment 2: Sedated (mm)		Average change (mm)
	Day 0	Day 2	Day 0	Day 2	
A	15.0	7.2	9.6	7.6	—
A	12.8	12.4	—	—	—
A	—	14.4	5.5	6.1	—
A	11.4	—	7.8	6.6	—
Mean $\pm$ SD	13.1 $\pm$ 1.8	11.3 $\pm$ 3.7	7.6 $\pm$ 2.1	6.8 $\pm$ 0.7	-2.2
B	—	—	9.2	8.2	—
B	—	—	5.1	6.3	—
B	—	—	6.8	6.1	—
B	—	—	6.3	6.3	—
Mean $\pm$ SD	—	—	6.8 $\pm$ 1.7	6.7 $\pm$ 1.0	-0.2
C	12.0	—	10.0	—	—
C	7.3	—	4.9	0	—
C	19.8	16.0	6.3	7.2	—
C	16.8	13.0	6.9	7.2	—
C	7.5	10.5	6.3	6.5	—
C	21.3	—	—	—	—
Mean $\pm$ SD	14.1 $\pm$ 6.1	13.2 $\pm$ 2.7	6.9 $\pm$ 1.9	5.2 $\pm$ 3.5	-1.1

Group A received 10 ml/kg of intraperitoneal dextrose (controls). Group B received 7.5 ml/kg of intravenous Macrodex and 5 ml/kg of intraperitoneal 10% dextrose only in experiment 2. Group C received 10 ml/kg of intraperitoneal Hyskon.

the fibrin plates to detect direct effects on plasminogen activator activity. Plates were also prepared containing 1% to 5% (w/v) dextran 70 (Sigma D-1390) to determine whether dextran present during clotting might alter the "lysability" of the fibrin clots, a property which has also been attributed to dextran.¹⁰

Blood samples were obtained by cardiac puncture in both experiments. Animals in the second experiment received 20 mg/kg of intramuscular ketamine 30 minutes before blood samples were drawn, whereas animals in the first experiment received ketamine only before operation.

Results

Intravascular plasminogen activator. The data comparing the lytic ability of pretreatment and posttreatment blood euglobulin fractions are presented in Table I. (Two sets of measurements were not available when animals died or when obvious experimental errors were made.) It was apparent that at 48 hours there was no significant change in plasminogen activator attributable to treatment with either intraperitoneal dextran (average change in lytic zone diameter = -2.2 mm) or intravenous dextran (average change in lytic zone diameter = -0.2 mm) compared with control values (average change in lytic zone diameter = -1.1 mm). An additional finding was that the euglobulin fractions of blood samples obtained from sedated rabbits in the second experiment had much less lytic activity than those obtained from alert rabbits in the first experiment ($p < 0.005$). The investigator's blood euglobulin frac-

tion, which had been assayed at the beginning of the experiment, was rechecked and found to be essentially unchanged, suggesting that there was no error in methodology. Euglobulin fraction samples produced slight lysis on heated plates (usually about 1% to 5% of the activity seen on unheated plates), indicating that practically all of the lytic activity was attributable to plasminogen activator.

Plasminogen activator activity of untraumatized peritoneum. Only data from the second experiment are presented (Table II) because, although unintentional, the design of the first experiment probably produced mostly traumatized samples. Mean diameters of the lytic zones produced by cecal samples from Hyskon-treated (23.3 ± 3.6 mm), Macrodex-treated (22.9 ± 8.2 mm), and control rabbits (23.5 ± 9.0 mm) were not appreciably different. Neither traumatized nor untraumatized parietal peritoneum produced lysis in either experiment. In all cases samples failed to produce lysis on heated plates, indicating that lytic ability was attributable to plasminogen activator.

Plasminogen activator activity of traumatized peritoneum. Data from experiment 1 yielded such wide variation that means between dextran-treated and control rabbits could not be compared. However, there was a significant ($p < 0.01$) difference in the number of samples from the two treatment groups that were incapable of producing a zone of lysis on the fibrin plates (Table III). Three of 34 (8.8%) cecal biopsy specimens harvested from rabbits treated 48 hours earlier with intraperitoneal dextran yielded no lysis while eight of

Table II. Average diameters of lytic zones produced by cecal biopsy specimens in experiment 2

Group	Diameter (mm)		
	Unabraded cecum	Abraded cecum	Change
A	28.9	27.7	-1.2
A	19.4	13.9	-5.5
A	26.5	22.0	-4.5
A	19.0	16.4	-2.6
Mean $\pm$ SD	23.5 $\pm$ 9.0	20.0 $\pm$ 6.2	-3.5
B	29.3	27.8	-1.5
B	14.8	13.9	-0.9
B	27.0	24.0	-3.0
B	20.5	14.4	-6.1
B	22.8	23.3	+0.5
Mean $\pm$ SD	22.9 $\pm$ 8.2	20.7 $\pm$ 9.0	-2.2
C	26.0	27.5	+1.5
C	20.7	32.2	+12.2
C	23.7	23.2	-0.5
C	23.7	0	-23.7
Mean $\pm$ SD	23.3 $\pm$ 3.6	20.7 $\pm$ 14.5	-2.6

Group A received 10 ml/kg of intraperitoneal dextrose (controls). Group B received 7.5 ml/kg of intravenous Macrodex and 5 ml/kg of intraperitoneal 10% dextrose. Group C received 10 ml/kg of intraperitoneal Hyskon.

21 (38%) samples from control rabbits were unable to cause lysis. Even though there were more female rabbits in the control group, there was no significant difference attributable to sex between lytic and nonlytic samples.

While the "unintentional" sampling trauma in the first experiment was quite capable of causing total depression of peritoneal plasminogen activator, the intentional abrasion trauma in the second experiment was not (Table II). Mean diameters of lytic zones declined minimally after abrasion trauma in all groups (3.5 mm in controls, 2.2 mm in Macrodex-treated rabbits, and 2.6 mm in Hyskon-treated rabbits). In one rabbit that received intraperitoneal dextran, there was a total depression of all three traumatized samples gathered. The surgery log contained the notation that the area of cecum that was the source of these samples appeared "bruised" so it appears likely that excessive trauma was present in this case.

Direct lytic ability of Hyskon. Drops of buffer, Hyskon, Macrodex, and 10% dextrose were also incubated on heated and unheated fibrin plates. Only the Hyskon drops caused lysis on the unheated fibrin plates (mean 9.7 ± 0.6 mm diameter) indicating some direct ability to activate plasminogen.

Lysis on plates containing dextran. There was no difference in the lytic zones produced by identical euglobulin fraction or urokinase samples incubated on standard fibrin plates and plates prepared with dextran 70 (1% to 5%, w/v) added before clotting.

Table III. Frequency of zero-diameter lytic zones in experiments 1 and 2

Group	Unabraded cecum	Abraded cecum	Total
Experiment 1			
A	5/9	3/12	8/21
C	2/17	1/17	3/34
Experiment 2			
A	0/24	0/12	0/36
B	0/30	1/15	1/45
C	0/24	3/12	3/36

Group A received 10 ml/kg of intraperitoneal dextrose (controls). Group B received 7.5 ml/kg of intravenous Macrodex and 5 ml/kg of intraperitoneal 10% dextrose. Group C received 10 ml/kg of intraperitoneal Hyskon.

Standardization of plates. The mean diameter produced by one unit of urokinase incubated on 49 different plates from nine batches of fibrinogen solution was 32.2 ± 1.5 mm.

Ascites, pleural effusion, death. Twelve rabbits received intraperitoneal Hyskon according to either experiment 1 or experiment 2 protocol. An additional rabbit (No. 13) received intraperitoneal Hyskon between the two experiments and was included with the pooled data (Table IV) pertaining to the frequency of ascites, pleural effusion, and death in Hyskon-treated rabbits. The expected ascites was observed in 5 of the 13 rabbits treated with intraperitoneal Hyskon. Ascites was minimal (approximately 15 ml) in three rabbits and greater than 60 ml in two rabbits.

Pleural effusions ranging from 15 to >100 ml (mean 50 ml) were also observed in eight of the 11 Hyskon-treated rabbits examined for this finding. Effusions were not expected and not checked for until midway through the first experiment. Of the 13 Hyskon-treated rabbits, five were also noted to have either dark arterial blood or respiratory distress. Three Hyskon-treated rabbits died between 24 and 48 hours after treatment and two died during the harvest of cecal samples. All had pulmonary effusions at necropsy. No rabbits treated with either intravenous Macrodex or intraperitoneal 10% dextrose had ascites or pleural effusion ($p < 0.005$), nor were there any perioperative deaths ($p < 0.05$).

Comment

Intravascular fibrinolysis. No effect on lytic ability of the euglobulin fraction of blood was detectable at 48 hours after treatment. The design of this study precluded assay until 48 hours, and it may be that this interval was too long to allow detection of an effect on plasminogen activator. In addition, a lack of effect on plasminogen activator does not rule out an effect on plasminogen activator inhibitors. Since the assay used

Table IV. Frequency of ascites, pleural effusion, respiratory distress, and death in Hyskon-treated rabbits

<i>Animal No.</i>	<i>Ascites (ml)</i>	<i>Pulmonary effusions</i>	<i>Respiratory symptoms</i>	<i>Death</i>
1	No	Not checked	No	No
6	15	Yes, not quantitated*	Gasping	24 hr
7	No	Not checked	Dark arterial blood	No
8	No	Bilateral, >80 ml	Gasping and dark arterial blood	Intraoperative
9	No	Bilateral, >15 ml	No	Intraoperative
11	No	No	No	No
13	No	No	No	No
16	15	Right, 65 ml	Labored breathing	Between 24 and 48 hr
19	60	Right, 65 ml; left, 40 ml	No	24 hr
22	15	Right, 30 ml; left, 15 ml	Respiratory distress	No
25	No	No	No	No
27	No	Right, 20 ml; left, 15 ml	No	No
28	60	Right, 15 ml	No	No

*The necropsy was done with the expectation that cardiac puncture-related trauma was responsible for the death. Instead, a large, pale pink pulmonary effusion was found, which spilled out of the pulmonary cavities before it could be quantitated.

here operates on the premise that activators are precipitated without inhibitors, a decrease in inhibitor that could be significant in vivo would not be detected. A time-course study with the use of more precise assays of plasminogen activators and inhibitors is necessary to properly evaluate the effects of intraperitoneal dextran on the intravascular fibrinolytic system.

In these experiments, dextran present during clotting of the fibrin plates did not affect lytic ability of identical samples. In vitro dextran has been shown to alter fibrin structure in such a way as to increase its lysability.¹⁰ The results of this study are in agreement with others,⁸⁻¹¹ which have shown that although in vitro dextran does alter fibrin structure, significant increases in lysability of clots are seen only after the infusion of dextran. This enhanced lysability is due to dextran's diminution of plasminogen activator inhibitors.

An interesting and unexpected finding was the marked depression of fibrinolytic activity in the euglobulin fraction of blood obtained from sedated rabbits. While this could be a pharmacologic effect of the ketamine itself, it is more likely related to the "stress-induced" release of plasminogen activator.⁸ Plasminogen activators and inhibitors are intimately involved in the resolution of venous thromboses.¹² If sedation can truly cause a reduction in intravascular fibrinolytic activity, one might legitimately question whether there is a link between the degree of perioperative analgesia and/or anesthesia and postoperative thromboembolic complications.

Peritoneal fibrinolysis. The results indicate that the plasminogen activator activity of untraumatized or abraded peritoneum was not augmented by either intraperitoneal or intravenous dextran. The failure of abrasion to severely depress fibrinolysis in this study most likely indicates that the trauma was not sufficiently severe. In retrospect, a standardized clamping or ischemic trauma would probably have been more successful

in achieving the expected depression of fibrinolysis. However, assuming that the harvesting technique in the first experiment did deliver severe crushing trauma to peritoneum of both experimental and control rabbits, it appears that intraperitoneal dextran offered significant protection against trauma-mediated depression of fibrinolysis. Since the severity of plasminogen activator depression has been correlated with postoperative adhesion formation, the effectiveness of Hyskon could be partly due to this preservation of plasminogen activator in traumatized peritoneum.

This assay could not distinguish effects on plasminogen activator or plasminogen activator inhibitors (that is, a zero zone of lysis could be due to either less plasminogen activator or more plasminogen activator inhibitor). However, since it has been shown that severely traumatized peritoneum inhibits the ability of normal peritoneum to cause lysis,⁵ the protection apparently offered by Hyskon most likely involves alteration or diminution of trauma-induced plasminogen activator inhibitors.

In agreement with the earlier observation of Myhre-Jensen et al.¹³ of plasminogen activator being absent in rabbit parietal peritoneum, this peritoneum in our study was unable to cause lysis of fibrin. In contrast, parietal peritoneum from other species does activate plasminogen.^{5, 7, 13}

Besides enhancing the ability of traumatized visceral peritoneum to effect lysis, Hyskon was able to directly activate plasminogen. Its presence in the peritoneal cavity in the immediate postoperative period must certainly enhance the lysis of the early fibrinous adhesions. Knowing whether these early adhesions form or are prohibited by the siliconizing or hydroflotation effects of dextran is a key to understanding whether the fibrinolytic properties demonstrated here are involved in the efficacy of Hyskon.

It is still not known why some patients are more

prone to develop adhesions than others. Ashby,¹⁴ in 1969, noticed a clustering of fibrotic lesions in patients with recurrent obstructing adhesions, suggesting some disorder of fibrinolysis. It has been observed that dextran seems to be most effective when preexisting adhesions are most severe.⁴ It is within reason to speculate that such patients might have a fibrotic tendency. If dextran does work mostly by virtue of its enhancement of fibrinolysis, then it may be a useful adjunct for treating the "fibrotic diathesis" hypothesized by Ashby.¹⁴

Complications of dextran use. Finally, the extremely high incidence of pulmonary effusions, respiratory distress, and death must be addressed. Dextran is considered a relatively safe compound. "Pseudo-Meigs' syndrome" of pulmonary effusion and ascites is thought to be a rare complication after use of excessive amounts of intraperitoneal Hyskon.¹ The ascitic fluid is thought to reach the pleural space either by diaphragmatic fenestrations or by lymphatic channels. Using a much less hypertonic solution than Hyskon, Adoni et al.¹⁵ found pulmonary effusions in 12 of 25 women treated with 400 to 500 ml of 10% dextran, with a molecular weight of 40,000, in normal saline solution (Rheomacrodex, Pharmacia) and concluded that the effusions they saw were benign. However, since they were using a much less hypertonic solution, direct extrapolation of these results to the use of Hyskon becomes difficult. Symptomatic pulmonary effusions in humans after 200 ml of Hyskon have been observed.² Tachypnea, chest pain, and tachycardia were also observed¹ and attributed to "diaphragmatic irritation" but could just as easily have been due to pulmonary effusion. Other animal experiments have noted unexplained mortality in Hyskon-treated animals.¹⁶ In light of the occurrence of pulmonary effusions, respiratory compromise, and death observed in this study, a similar association could have been present but undetected in those experiments.

The frequency and severity of pulmonary effusions related to Hyskon use in humans could easily be investigated. Firmly establishing the "benignity" of this side effect is desirable for a drug that will probably be used more and more as the field of infertility surgery expands.

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Premature rupture of the membranes before 28 weeks: Conservative management

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The maternal and fetal outcomes of 70 consecutive singleton pregnancies with prolonged premature rupture of the membranes before 28 weeks, which were managed expectantly, were evaluated; 58.6% of the mothers developed chorioamnionitis and 13% postpartum endomyometritis. No long-term maternal sequelae were noted. None of the 22 infants born before 25 weeks survived the neonatal period. The weights of the 35 surviving infants ranged between 800 and 4100 gm. Only 29.4% of the 17 infants with long-term follow-up were both physically and developmentally normal at 6 months of age or more. (AM J OBSTET GYNECOL 1986;155:471-9.)

Key words: Premature rupture of membranes, chorioamnionitis, maternal morbidity, long-term survival, neonatal morbidity

Until recently, premature rupture of the membranes before the twenty-eighth week of gestation often resulted in the immediate termination of pregnancy by the clinician. With the significant improvement in neonatal care of the extremely premature infant, a more conservative approach is being followed. In a retrospective study Taylor et al.¹ reported a neonatal survival rate of 24.5% in 53 cases of premature rupture of the membranes between 16 and 25 weeks of gestation. However, 41% of the mothers developed chorioamnionitis, and there was one case each of sepsis and pelvic thrombophlebitis. Of the surviving infants that were followed, 56% were developmentally normal.

Other potential risks in the expectant management of prolonged rupture of membranes include fetal distress, intrauterine growth retardation, pulmonary hydrops, and fetal deformities.²⁻⁴

The aim of this study is to evaluate the merits of expectant management of pregnancies with prolonged rupture of membranes (>24 hours) that occurred before 28 weeks' gestation.

Material and methods

Starting in July 1978 and at the patient's request, 70 consecutive singleton pregnancies at the University of Miami, Jackson Memorial Hospital, between 20 and 27 completed weeks of gestation with confirmed pre-

ture rupture of the membranes for more than 24 hours were followed prospectively.

The dates were confirmed by ultrasound evaluation. In addition to the sonographic findings, premature rupture of the membranes was confirmed by a combination of visualization of amniotic fluid in the vagina as well as a positive Nitrazine and/or fern test on the fluid.

Management consisted of hospitalization with bed rest and no pelvic examinations. In addition, the patients were monitored for the development of chorioamnionitis. If any two of specific criteria (fever $\geq 100.4^{\circ}$ F, progressive leukocytosis, uterine tenderness or irritability, fetal tachycardia >180 bpm, or foul-smelling amniotic fluid) developed, the presumptive diagnosis of chorioamnionitis was made and delivery achieved. Labor was also induced if fetal death occurred. If spontaneous labor began even in the absence of infection, the patient was allowed to deliver. No tocolytic agents or steroids were used, and no amniocentesis or fetal lung maturity studies on the vaginal pool amniotic fluid were undertaken. Most patients with chorioamnionitis were given broad-spectrum antibiotic coverage in labor or before a cesarean section. All patients remained in the hospital until delivery was achieved or until amniotic fluid leakage stopped for 48 hours after ambulation in the hospital.

Results

The maternal ages of the 70 patients ranged from 14 to 40 years with a mean age of 26.6 years and a median age of 25 years. The racial distribution was as follows: 17 (24.3%) were white, 39 (55.7%) were black, nine (12.9%) were Hispanic, and five (7.1%) were Haitian.

Nine of the 70 patients (12.9%) were primigravid,

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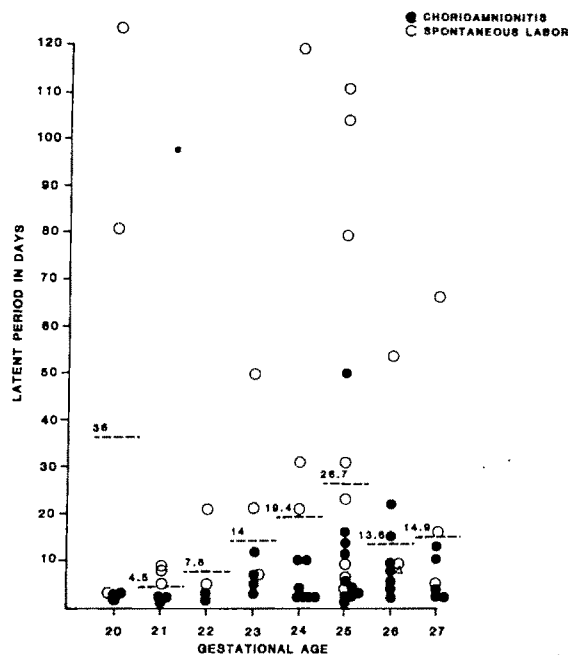


Fig. 1. Relationship between the stage of gestation, the latent period, and the development of chorioamnionitis.

and 42 (60%) had at least one (one to six) previous term gestations. Only 18 (25.8%) of the patients gave a completely negative history in the current or previous pregnancies. The remaining histories included one or more of the following: in 17 patients (24.3%) the preceding one to three pregnancies ended in premature labor; 20 patients (30.0%) gave a history of one to six spontaneous abortions in the pregnancies preceding the current one.

Thirty-two (45.7%) of the patients reported a history of cervical or myometrial operations immediately before the current pregnancy; 21 (30.0%) of the patients had a first-trimester termination of pregnancy, five (7.1%) a diagnostic dilatation and curettage, two (2.9%) a cervical cone biopsy, three (4.3%) a trachelorrhaphy, and one (1.4%) a uterine reconstruction procedure.

During the current pregnancy five (7.1%) had a cerclage procedure, two (2.9%) became pregnant with an intrauterine device in situ that could not be removed earlier, and one (1.4%) had large fibroids. Twenty (28.6%) patients smoked more than 10 cigarettes per day and 11 (15.7%) were drug and/or alcohol abusers, two conditions that may predispose women to premature rupture of the membranes.³ A history of recent coital activity was not known. Seven (10.0%) of the patients presenting had vaginal bleeding with the premature rupture of the membranes. The bleeding was not severe enough to warrant immediate termination of the pregnancy.

The gestational age distribution at the onset of pre-

Table I. Distribution of gestational age at onset of premature rupture of the membranes

Completed week of gestation	No. of patients
20	6
21	6
22	4
23	7
24	10
25	18
26	11
27	8
Total	70

mature rupture of the membranes is depicted in Table I. The mean was 24.1 weeks and the median 25 weeks.

Latent period. One patient who presented at 26 weeks of gestation left the country undelivered 3 days after fluid leakage stopped. The length of the latent period between membrane rupture and delivery in the remaining 69 patients ranged between 2 and 124 days with a mean of 19.1 days and a median of 7.5 days.

No correlation was observed between the latent period and gestational age at time of premature rupture of the membranes (Fig. 1). Of the 69 patients, 35 (50.7%) were delivered within 1 week, 49 (71.0%) within 2 weeks, and 57 (82.6%) within 1 month. In only 12 (17.4%) patients was the latent period longer than 1 month.

The reasons of the delivery were as follows: 54 (78.3%) patients went into spontaneous labor, 26 had chorioamnionitis, and 28 were without any clinical evidence of infection. In an additional 13 patients in whom chorioamnionitis was present, labor was induced. No inductions were performed for fetal death without chorioamnionitis. No failure of inductions occurred. Two patients had a cesarean section without either chorioamnionitis or labor: one for fetal distress at 28 weeks and the other for a breech presentation at 27 weeks in which the amniotic fluid became meconium stained.

In 10 of the 70 patients (14.3%) the fluid stopped leaking when they were in the hospital. They were discharged from the hospital 48 hours after ambulation without evidence of further leakage. One of these patients left the country, and the pregnancy could not be followed. A summary of the remaining nine patients is presented in Table II. Fluid leakage stopped between 4 and 50 days after the onset of premature rupture of the membranes, with a mean of 15.1 days and a median of 10 days. Cessation of fluid leakage until the onset of labor lasted for 6 days or less in three of the nine patients. One developed clinical chorioamnionitis, and one went into spontaneous labor 3 days after the leakage stopped. The third presented 6 days later with abruptio placentae necessitating delivery. The other six

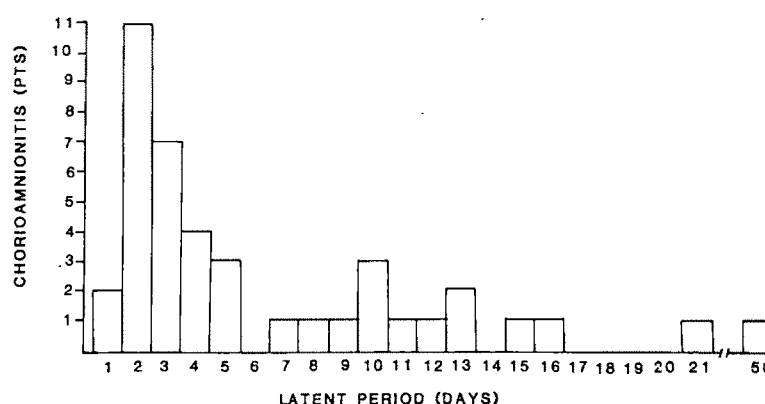


Fig. 2. Onset of chorioamnionitis in relation to latent period.

Table II. Patients in whom fluid leakage stopped (n = 9)

Gestational age at premature rupture of membranes (wk)	Leakage stopped		Gestational age at delivery (wk)	Birth weight (gm)	Outcome
	Days after onset	Days until start of labor			
20	7	117	38	3040	Normal
20	5	76	32	1760	Normal
24	50	70	41	3600	Normal
25	13	3	27	1240	Normal
25	23	57	36	2725	Normal
25	6	99	40	4100	Normal
27	10	6	29	1220	CLD
27	4	3	28	1000	NEC
27	18	49	37	2200	Normal

CLD = Chronic lung disease; NEC = Necrotizing enterocolitis.

patients carried their pregnancies for 49 to 117 days more and delivered infants weighing between 1760 and 4100 gm at 32 to 41 weeks of gestation.

Mode of delivery. All 22 (31.9%) deliveries of <25 weeks of gestation (for which at University of Miami, Jackson Memorial Hospital, there is no reasonable chance of survival—<20%) were delivered vaginally. Of the 47 patients who were delivered beyond 25 weeks, 12 (25.5%) were delivered by cesarean section. The indication for the cesarean section in 10 patients was for breech presentation (two also had a previous cesarean section). The other two had vertex presentation; one had a previous metroplasty and the other fetal distress before labor.

Chorioamnionitis. Of the 70 patients 41 (58.6%) developed clinical evidence of chorioamnionitis; 26 (63.4%) of these patients went into spontaneous labor, and 13 (31.7%) had to be induced. The other two patients (4.9%) underwent a primary cesarean section, one for breech presentation at 32 weeks and the other for previous metroplasty. The incidence of chorioamnionitis peaked in the second day and decreased gradually to the fifth (Fig. 2). Twenty-seven of the 41 patients (65.9%) developed the infection in the first 5 days.

From the sixth day after membrane rupture the incidence of chorioamnionitis remained constant until about the sixteenth day. Only two patients developed the infection after the sixteenth day, one at day 21 and the other at day 50. No distinct characteristics were observed in those two patients. Most patients with established clinical chorioamnionitis were started on broad-spectrum antibiotics when in labor, before an elective cesarean section, or in the postpartum period.

Maternal morbidity. Nine (13.0%) of the 69 patients developed postpartum endomyometritis, with five of them following a cesarean section. Of the four patients that developed the infection following a vaginal delivery all had chorioamnionitis before delivery. However, only two were receiving antibiotics. One such patient, in addition, developed postpartum hemorrhage necessitating curettage.

Six patients (8.7%) had a retained placenta. In three it was removed manually, and in the other three a curettage was necessary. Other maternal complications included two abruptio placentae (with vaginal delivery without any sequelae), and one each of postpartum hemorrhage, post-cesarean section atelectasis, wound infection, and cervical laceration. One patient with a

Table III. Neonatal deaths occurring after first day of life

<i>Gestational age at delivery (wk)</i>	<i>Birth weight (gm)</i>	<i>Apgar score (1,5 min)</i>	<i>Age at death (days)</i>	<i>Cause of death</i>
24	560	7,7	17	Respiratory distress syndrome, necrotizing enterocolitis
25	960	1,2	9	Respiratory distress syndrome, seizure Pneumonia
26	1020	7,8	2	Respiratory distress syndrome, intraventricular hemorrhage
27	1180	1,5	3	Respiratory failure
27	1280	2,2	4	Respiratory distress syndrome, seizure Pneumonia
27	1000	6,8	60	Necrotizing enterocolitis, Pulmonary edema
28	1630	5,7	5	Multiple congenital anomalies

Table IV. Neonatal morbidity in the 35 surviving infants*

	<i>n</i>	<i>%</i>
Respiratory distress syndrome	15	42.9
Central nervous system bleeding	10	28.6
Subependymal	5	
Intraventricular	5	
Hydrocephalus	10	28.5
Associated with central nervous system bleeding	8	
Not associated with central nervous system bleeding	2	
Anemia	9	25.7
Intermittent apnea	8	22.9
Patent ductus arteriosus	7	20.0
Bronchopulmonary dysplasia	4	11.4
Persistent fetal circulation	4	11.4
Sepsis	4	11.4
Necrotizing enterocolitis (NEC)	3	8.6
NEC needing bowel resection	2	
Retrolental fibroplasia	3	8.6
Other complications	20	57.1

*Some infants had more than one complication.

previous cesarean section had a placenta accreta at 26 weeks necessitating an abdominal hysterectomy. Her postoperative course was benign.

Perinatal outcome. Of the 69 infants delivered 32 (46.4%) were male and 37 (53.6%) were female.

Fifteen babies (21.7%) were stillborn. In 19 deliveries (27.5%) the infants died in the neonatal period, giving a perinatal mortality rate of 49.3%. Thirty-five newborn infants left the nursery. Hence the immediate neonatal survival rate was 50.7%.

Of the 15 stillborn infants 13 (86.7%) were born before gestational week 26. Their birth weights ranged between 200 and 900 gm, with a mean of 531.1 gm and a median weight of 457.5 gm. Of the other two, one was born at 32 weeks with a birth weight of 1247 gm and died of undiagnosed prolapsed cord and the other at 28 weeks (1180 gm) following severe chorioamnionitis in the mother.

Of the 19 neonatal deaths 12 (63.2%) occurred within hours of birth. The gestational ages at delivery of all those 12 infants were <27 weeks, and their birth weights ranged between 400 and 1300 gm. They all had Apgar scores of ≤ 3 at 5 minutes, and all died of extreme prematurity, respiratory insufficiency, and/or sepsis. Seven of the neonates survived more than 24 hours. The characteristics of these infants and the cause of death are listed in Table III.

Thirty-five infants (50.7%) survived the immediate neonatal period and left the nursery alive. None of those were delivered before the twenty-fifth week of gestation. The birth weights of the survivors ranged between 800 and 4100 gm with a mean birth weight of 1850 gm and a median weight of 1220 gm. The hospital stay of these infants ranged between 2 and 281 days with a mean of 63.9 and a median of 50 days. Neonatal survival was related to the gestational age at delivery

Table V. Long-term sequelae of 12 surviving infants*

	n	%
Chronic lung disease	7	41.2
Hydrocephalus	3	17.7
Somatic growth failure	2	16.7
Developmental abnormalities	9	75.10
Bayley Score <68 (abnormal)†	5	
Bayley Score 68-84 (questionable)	4	
Cerebral palsy	1	8.3
Severe hearing loss	1	8.3
Speech problem	1	8.3
Hyperactivity	1	8.3

*Some infants have more than one abnormality.

†Abnormal Bayley Score is <100.⁶

rather than the gestational age when the membranes were ruptured (Fig. 3).

None of the six infants with a birth weight >1760 gm developed any major complications in the neonatal period. The most common neonatal complication in the remaining 29 survivors was hyperbilirubinemia that occurred in 24 infants; 21 of them were given phototherapy, and three needed at least one exchange transfusion. The other significant complications are enumerated in Table IV.

Long-term outcome. Of the 35 surviving infants, 17 (48.6%) were followed after being discharged from the nursery for a period of 6 months to 6 years. Only two of the infants weighed more than 2500 gm at birth (3240 and 2725 gm), and both are reported to be physically and developmentally normal at 3 years of age. The birth weights of the remaining 15 infants ranged between 800 and 1520 gm. Three babies that weighed between 1130 and 1520 gm also had normal growth and development up to 2 years of age, giving a total incidence of 29.4% normalcy (5 of 17 infants). The long-term physical and developmental abnormalities (including Bayley scores⁶) in the remaining 12 infants with long-term follow-up are depicted in Table V.

Comment

In this study the outcome of 70 pregnancies with prolonged premature rupture of the membranes between 20 and 27 completed weeks of gestation that were managed expectantly were evaluated. Since multiple gestations are known to be predisposed to premature rupture of the membranes⁶ and since they are associated with other complications,⁷ only singleton pregnancies were included in order to limit the number of variables.

The age distribution of the patients in the study was similar to that of the general gravid population cared for at University of Miami, Jackson Memorial Hospital. The ratio of blacks to whites (3.3:1) and blacks to Hispanics (4.3:1) in the study group was higher than the

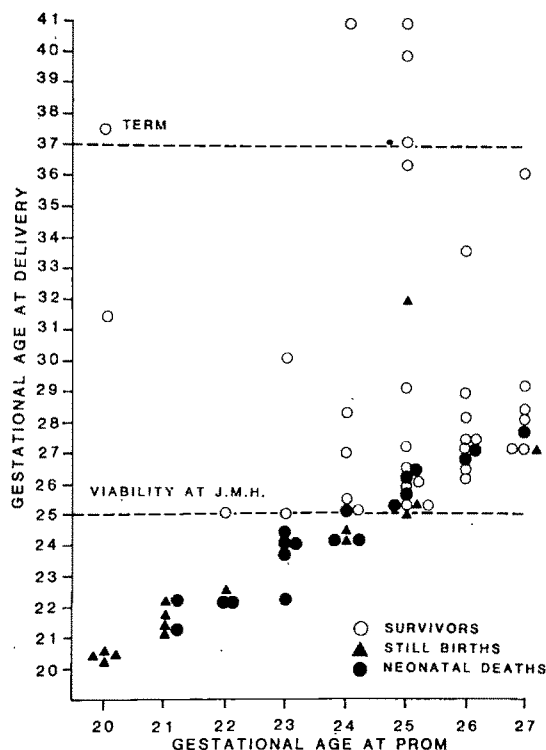


Fig. 3. Relationship between gestational age at premature rupture of the membranes (PROM) and gestational age at delivery with fetal survival.

approximately 1:1 ratio in the general obstetric population in the same institution. This observation is similar to that reported by Hendricks,⁸ and for which no explanation can be offered at the present time.

Etiology. Only one quarter of the patients have an apparently negative history. In the remainder, one or more predisposing factors for premature rupture of the membranes^{2,9} could be demonstrated including a poor obstetric history in more than half (54.3%). An interesting observation is that 21 (30%) of the patients that presented with premature rupture of the membranes in this study gave a history of at least one first-trimester elective termination of pregnancy in gestations immediately preceding the current one. More than 70% of those patients with this history of termination of pregnancy had completely negative histories before or following the procedure, including 10 with one or more previous term pregnancies and five in whom the termination of pregnancy was performed in the first pregnancy. Although not statistically analyzed, the 30% incidence of termination of pregnancies in the study population seems to be higher than the 10% incidence per total deliveries at University of Miami, Jackson Memorial Hospital. Large-scale studies to reevaluate the probable causal relationship between termination of pregnancy and premature rupture of the membranes should be conducted. Infection of the cho-

tion and amnion as a cause of premature rupture of the membranes has been proposed by Minkoff.¹⁰ It is unlikely that this is a major contributor to membrane rupture in this study, since all the cases of chorioamnionitis that developed had occurred more than 24 hours after premature rupture of the membranes.¹¹

Latent period. In contrast to the observations of Taylor and Garite¹ and Wilson et al.¹² in the very premature gestation, this study did not show any inverse relationship between the gestational age at membrane rupture and the latent period. It was interesting to note, however, that the infants' ultimate survival was influenced by the gestational age at delivery rather than at the time of premature rupture of the membranes. Ten infants (>30%) of the 33 patients whose membrane rupture occurred before reasonable viability is reached at University of Miami, Jackson Memorial Hospital (≥ 25 weeks) survived the neonatal period. Only one infant in this group, who weighed 1220 gm at birth, developed chronic lung disease, and another weighing 1000 gm had necrotizing enterocolitis that was successfully treated surgically and left the nursery in excellent condition. The rest had a completely uneventful neonatal course and are presumed to have remained normal. These observations suggest that the best neonatal outcome and long-term prognosis of the infant occur in the pregnancies with premature rupture of the membranes in which fluid leakage stops for a considerable period of time.

Chorioamnionitis. Chorioamnionitis developed in 58.6% of the patients. This seemingly high incidence of infection may in fact be falsely elevated because of the exclusion from the study of those patients with premature rupture of the membranes who delivered in the first 24 hours of membrane rupture when the incidence of chorioamnionitis in that group of patients is extremely low. The role of prophylactic antibiotics in preventing chorioamnionitis is being investigated.

In 65.9% of the patients that developed chorioamnionitis the infection occurred in the first 5 days of premature rupture of the membranes. This incidence decreased significantly to 11.8% (two of 17 patients) after a latent period of 16 days was reached. The remaining 15 patients went into spontaneous labor and delivered vaginally except for four that underwent a cesarean section for breech presentation. These observations suggest that elective induction of labor when "reasonable" fetal maturity is reached on the grounds of preventing chorioamnionitis may not be advisable, since the conservative approach of awaiting spontaneous labor at least in theory seems to decrease their incidence of abdominal deliveries secondary to failed induction. An elective cesarean section, however, for obstetric or fetal indications (that is, breech presenta-

tion) may still be necessary when it is deemed by the obstetrician that fetal maturity is reached.

Chorioamnionitis is known to predispose the patient to developing endomyometritis in the postpartum period. In this study only five of the 41 patients (12.2%) with chorioamnionitis developed the postpartum infection. This relatively low incidence may in part be due to the expeditious delivery of the patients as soon as the diagnosis of chorioamnionitis is made. Furthermore, it was observed that intrapartum broad spectrum antibiotic coverage in this series of patients with chorioamnionitis reduced the incidence of postpartum endomyometritis from 15.8% to 9.1%. All cases of endomyometritis were treated successfully and left the hospital in <6 days with no apparent long-term sequelae.

Although chorioamnionitis does not seem to present any long-term effects on the mother, there are two major concerns for the neonate: (1) neonatal sepsis¹³ and (2) the long-term mental and physical development of the infant.¹⁴ In this study, four infants (5.8%) developed neonatal sepsis, an incidence well in line with the 0.5% to 25% reported in the literature.¹⁵ All babies were successfully treated with no apparent long-term sequelae. The mother of two of the infants with sepsis had chorioamnionitis and two did not. This shows either that maternal infection of the amniotic cavity does not compound the effects of premature rupture of the membranes on neonatal infection or that the chorioamnionitis was underdiagnosed. As far as the long-term mental and physical development of the infants are concerned, this study did not show any association between chorioamnionitis in the mother and any long-term physical or mental handicap in the infant. It should be kept in mind, however, that the number of infants followed to ≥ 6 months of age was only 17.

Maternal morbidity. Expectant management of premature rupture of the membranes in this study resulted in a high incidence of maternal morbidity similar to what Taylor and Garite¹ found in their retrospective study; 58.6% of the mothers developed chorioamnionitis and 33.6% other postpartum complications. If one analyzes the complications, however, all were satisfactorily corrected without leaving any apparent long-term sequelae, and all patients were discharged from the hospital within a week of delivery. Only one patient needed a hysterectomy for a placenta accreta following two previous cesarean sections, a situation which obviously was not related to the expectant management.

Perinatal outcome. In contrast to other reports in the literature citing a preponderance of male fetuses in preterm premature rupture of the membranes,^{1, 15} the male-to-female ratio in this study is reversed (1:1.2).

All infants born before 25 weeks of gestation were either stillborn or died in the nursery. This implies that at University of Miami, Jackson Memorial Hospital the neonatal survival before 25 weeks is poor, and cesarean section delivery for fetal indications at that stage of gestation is probably not warranted, since the potential maternal complications arising from the procedure far outweigh the extremely small chance of fetal survival. The smallest infant to survive the neonatal period in this study was born at 25 weeks and weighed 800 gm. No attempt was made to compare the neonatal outcome as to the mode of delivery because (1) the sample of patients studied was small and (2) the mode of delivery was not prospectively randomized.

All the neonatal complications encountered were attributable to prematurity. Of the 54 infants born alive 19 (35.2%) developed the respiratory distress syndrome. All but eight were born before 27 weeks. In the eight infants born between 27 and 31 weeks the disorder was mild and they recovered completely except for one who died with associated pneumonia and seizures. Could maternal steroid administration have reduced the above incidence of significant respiratory distress syndrome? The benefit of maternal steroid therapy in reducing respiratory distress syndrome in premature rupture of the membranes is still controversial,¹⁵ and its administration before 27 weeks of gestation, especially with premature rupture of the membranes, was not found to be helpful in reducing the incidence of the disorder.¹⁶ Since most of the serious and fatal cases of respiratory distress syndrome in this study were delivered before gestation of 27 weeks and since all but one of the cases that developed the disorder after 27 weeks recovered completely, in all probability steroid administration to the mother in this study may not have improved the ultimate outcome.

The neonatal complication that probably had the most impact on the long-term outcome of the infants in this study was the development of intraventricular hemorrhage. Six of the nine infants with abnormal Bayley scores after 6 months of age developed the complication in the neonatal period. The occurrence of intraventricular hemorrhage in this study was more related to prematurity than to the mode of delivery. Research in prevention of this potentially serious complication is obviously warranted.

Pregnancies with prolonged periods of oligohydramnios are known to result in a tetrad of fetal complications. These are pulmonary hypoplasia, orthopedic deformities, Potter's facies, and intrauterine growth retardation.¹⁷ These potential problems prompted Perlman et al.¹⁸ to suggest that the incidence of the above complications increases significantly after 2 weeks of fluid leakage. More recently Nimrod et al.⁴ confirmed

the above observations in a retrospective case-controlled study. None of the infants in this study showed any facial or orthopedic deformities, and only one had intrauterine growth retardation. Four of the infants had questionable clinical evidence of mild pulmonary hypoplasia and survived the neonatal period; two of them continued to have chronic lung problems after discharge from the hospital, and the other two were lost to follow-up. No stillbirths or neonatal deaths occurred because of the disorder. This low incidence of the "oligohydramnios syndrome" in our series despite prolonged periods (up to 54 days) of fluid leakage is intriguing. A probable explanation is that despite the continuous leakage of amniotic fluid, no levels of oligohydramnios critical to causing the syndrome were reached. It is unfortunate that no follow-up sonographic examinations were used to demonstrate the remaining amniotic fluid volume.

In conclusion, the ultimate aim of a pregnancy is to deliver a healthy infant that develops normally, with minimal complications to the mother. This study shows that expectant management of premature rupture of the membranes before the twenty-eighth week of gestation does not contribute to long-term maternal morbidity. The long-term morbidity of the 17 infants that were followed, on the other hand, was discouraging because only five (29.4%) were physically and mentally normal at 6 months of age or more, despite prolonged periods of maternal and infant hospitalization. If in addition, one considers the total of 34 stillbirths and neonatal deaths that occurred in this series, the final infant normalcy rate at 6 months of age or more, which should be the ultimate goal of any pregnancy, approaches a meager 15%.

The results of this small-scale study are by no means conclusive of the merits and disadvantages of conservative management of premature rupture of the membranes in the very premature gestation. The addition of more data from various medical centers is indeed necessary to provide a better understanding of the problem and help the clinician formulate a rational approach to the management of this common and intriguing complication of pregnancy.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. EDGAR O. HORGER III, Charleston, South Carolina. This report involves a very conservative approach in the management of 70 consecutive singleton pregnancies with premature rupture of membranes between 20 and 27 weeks' gestation. There was no attempt to assess fetal lung maturity by amniotic fluid studies. No cultures were taken. Corticosteroids were not used to induce fetal pulmonary surfactant. Tocolytics were not used to stop premature labor, and no patients received prophylactic antibiotics. The management plan was one of simple watchful expectancy. Although I personally can support such a program, this report fails to document fully its appropriateness.

There really is no acceptable alternative to conservative management of this group of patients with mean gestational age of 24.1 weeks at membrane rupture. Prompt delivery is out of the question. There were no

surviving infants among the 22 who were delivered at 25 weeks' gestation or earlier. A major point in favor of conservative management is found in the fact that 10 of the 70 patients eventually stopped leaking amniotic fluid and were discharged from the hospital. At least five of these ultimately were delivered at 36 weeks or later.

Although there were 35 survivors among the 69 infants delivered, only five of the 17 infants followed for at least 6 months were physically and mentally normal. The report attempts to improve this long-term outlook by assuming that half of the 18 infants who were not followed also were developing normally. I feel that such an assumption is inappropriate and perhaps misleading.

The 21.7% incidence of stillbirths seems rather high, but no explanation for this is offered. Twelve of the 15 stillborn infants were delivered before 25 weeks' gestation. Were these antepartum or intrapartum fetal deaths? Was any form of fetal monitoring being used in these pregnancies? The 58.6% incidence of chorioamnionitis seems extremely high and warrants some explanation.

At a recent meeting I had the opportunity to question 17 obstetricians from around the United States regarding management of a patient with ruptured membranes at 24 to 25 weeks' gestation in an otherwise uncomplicated pregnancy. Each of these physicians chose a conservative approach. Only one doctor elected to assess fetal maturity through amniotic fluid tests, but 11 took cultures for group B streptococcus and/or gonococcus. Eight ordered ultrasonography to confirm gestational age and to assess amniotic fluid volume. Three ordered nonstress tests. One gave his patient corticosteroids in an attempt to induce fetal lung maturity, and two used prophylactic antibiotics. Six chose to use tocolytics when uterine contractions began 24 hours after membrane rupture. Surprisingly, only nine of these obstetricians expressed concern about subsequent fetal development in the face of oligohydramnios. I believe that this diversity of answers shows that conservative management has different meanings for different managers. No ideal protocol has been established, but we should be grateful to Dr. Beydoun for sharing his experience with us. I agree with his conclusion that more data are needed before the best management plan is known.

DR. H. LIPPARD, Lynchburn, Virginia. I would like to ask, just as a matter of basic science information, whether there is any evidence that there is in some patients a substantial accumulation of fluid which may be interpreted as amniotic fluid, and whether there is a substantial collection of fluid between the chorion and the amnion. I raised this question because I am sure all of us have had occasions when patients come in with unequivocal gushes of fluid, a Nitrazine test indicates that it is probably amniotic fluid, and a pelvic examination reveals a bulging of forewaters. We tend to call this, perhaps, a high leak. It has been my observation that such patients tend to do better than those who

have a gush of fluid and then labor ensues fairly promptly. I have no way of knowing whether there may be a separate subgroup of patients who are going to do well, and who have a collection of fluid between the amnion and chorion. My question is, Is there any evidence to indicate that some patients do have a substantial accumulation of fluid between the chorion and the amnion?

DR. WILLIAM J. LEMAIRE, Miami, Florida. I am not sure if it is appropriate to ask a question of a discussant but when Dr. Horger presented his tabulation of the 17 physicians that he interviewed, I think in order for us to appreciate what that means, we probably should know where these physicians were coming from and what the composition was of the 17 physicians.

DR. HORGER. I question whether it is appropriate to respond to that question; also, as a matter of fact, the 17 were candidates for certification at the oral examination of the American Board of Obstetrics and Gynecology last month.

DR. ROBERT C. CEFALO, Chapel Hill, North Carolina. To respond to the first questioner, I think that we do have evidence of chorionic leak, there is indeed an accumulation of forewaters, that there is such a thing as a high leak, that there does appear to be some protective function as opposed to a low leak, and that evidence is accumulated through ultrasound. We do have pictures showing us indeed that the rupture occurred in the amnion, that the chorion was intact, and that the fluid does accumulate. I would urge you to use ultrasonography for your patients with premature rupture of membranes, looking for that possibility and then put that into your computer when you decide on conservative management.

DR. BEYDOUN (Closing). As far as amniocentesis is concerned, first, this study was done in 1978 and it was

not yet an established protocol. Even now it is quite controversial, since more than 50% may not be able to get the amniotic fluid. As far as the treatment of giving steroids, because of the lack of time I did not explain the findings; but all of the patients that had severe respiratory distress syndrome were ≤ 27 weeks, and we all know now that at least the use of steroids in ruptured membranes is quite controversial. Furthermore, the collaborative study that was done in this country showed that in addition to ruptured membranes, it was not helpful to give steroids earlier than 27 weeks. In our study, all the patients with respiratory distress syndrome at 28 weeks or 27 weeks did well; the symptoms were mild. So in answer to your question, in this study most probably steroids may not have altered the ultimate outcome of these patients. Regarding antibiotics, there also is another controversial problem in giving prophylactic antibiotics, but I failed to mention that if a patient has any symptoms that suggest chorioamnionitis, she was placed on antibiotic coverage around the time of delivery and during the postpartum period.

As far as the use of culture is concerned, I did not include it because also at that time between 1978 to around 1980, we got very erroneous results. Most of them were mixed vaginal flora, and as a matter of fact, none of those patients had a positive group B culture. That is why I did not include it because it could have been a laboratory error at that time. As far as the tocolytics are concerned, I personally, and I am sure many others, believe that if we try to stop contractions in those patients after 24 hours of ruptured membranes, there is a very good chance that the uterine irritability or contractility might be secondary to a subclinical chorioamnionitis that we do not want to mask, and in which pregnancy should not be prolonged.

Factors affecting pregnancy rates in a donor insemination program using frozen semen

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The results of therapy of 226 women receiving frozen and fresh donor semen are reported. Overall, 45.6% of patients conceived with a monthly fecundability of 0.102. The effect of a variety of factors on fertility rates was analyzed in life-table analysis of 1000 cycles. Optimal pregnancy rates were obtained in couples with azospermia (0.17) or no female infertility factors (0.2). Patients with ovulatory dysfunction treated with clomiphene also had optimal pregnancy rates (0.17 per cycle). Endometriosis reduced fecundability significantly (0.04 per cycle). There was no significant difference in pregnancy rates per cycle between fresh (0.12) and frozen (0.09) semen. Acceptable pregnancy rates were obtained with frozen semen therapy and in patients with treated ovulatory dysfunction. (AM J OBSTET GYNECOL 1986;155:480-5.)

Key words: Infertility, donor insemination, frozen semen

Artificial insemination with donor sperm is a widely used treatment for male infertility. Treatment efficacy has been evaluated by a number of excellent life-table analyses of fecundability rate and its correlation with single factors, such as patient age,^{2,3} postcoital test,⁴ fresh versus frozen sperm,¹ and the effect of male and/or female factors on success rates with fresh semen.⁵

The present study was designed to evaluate a variety of factors, particularly fertility factors in the woman and their effect on fecundability in a donor program using primarily frozen donor semen. A preliminary analysis of the data from the program has been previously published.⁶

Methods

All patients participating in the program of artificial insemination with donor sperm at North Carolina Memorial Hospital from July 1, 1980, to July 31, 1984, were studied. Patients were identified by retrospective analysis of the records of the artificial insemination with donor sperm program. Special insemination forms recording cycle day, mucus quality, and type of specimen were in use for this period. Data from these forms were extracted and entered into a data analysis program on a Zenith minicomputer (Microstat statistical program).

Initial patient evaluation included physical exami-

nation, basal body temperature charts, and mid-luteal serum progesterone, prolactin, and testosterone levels. Hysterosalpingography was routinely performed and inseminations limited to patients with one or both tubes patent. Patients with anovulation, oligoovulation, or luteal phase dysfunction were treated with clomiphene to regulate cycles and to correct the midluteal phase progesterone level to >15 ng/ml as previously described.⁷ Patients who failed to conceive in six cycles routinely underwent laparoscopy.

Semen was obtained from healthy donors who had both genetic screening⁸ and biologic screening before acceptance.⁹ Donors screened before 1983 had total sperm counts $>80 \times 10^6/\text{ml}$ (mean = $111.4 \times 10^6/\text{ml}$), mean motility 70.4%, and motile sperm concentration $\geq 60 \times 10^6/\text{ml}$ (mean = $79 \times 10^6/\text{ml}$). Semen was frozen in glycerol in 1 ml aliquots in liquid nitrogen with a Linde BF-5 freezing core at 1° to 2°C per minute for 30 minutes and 8.5°C per minute subsequently. Semen was stored in liquid nitrogen. Specimens were defrosted immediately before insemination for 15 minutes in 4°C water and maintained at room temperature until used. Only semen samples with postthaw motility $\geq 50\%$ were inseminated. Fresh semen from the same donors was used when available and was entered as such for further analysis. One to three inseminations per cycle were used with timing based on cervical mucus quality and on basal body temperature charting in an attempt to identify the time of ovulation. A total of 0.2 ml of semen was placed in the external cervical os and the remaining 0.8 ml placed in a Mylex cervical cup placed on the cervix.

Pregnancies were confirmed by serum pregnancy testing and subsequent pregnancy follow-up was reported by the patients. No attempts were made to contact physicians.

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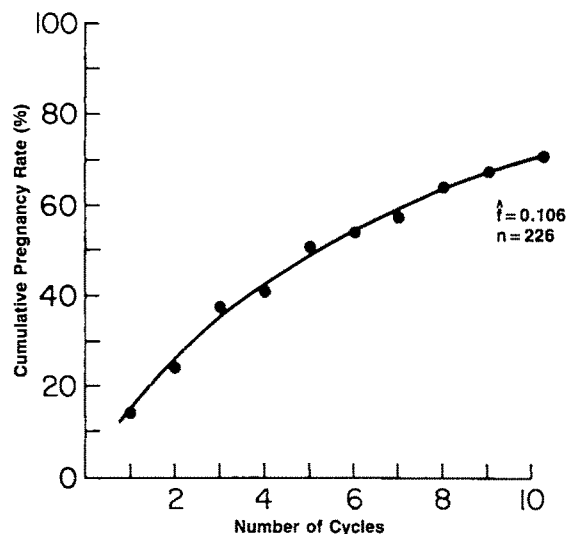


Fig. 1. Cumulative fecundability curve for 966 cycles of artificial insemination with donor sperm ($\hat{f} = 0.106$, $n = 226$).

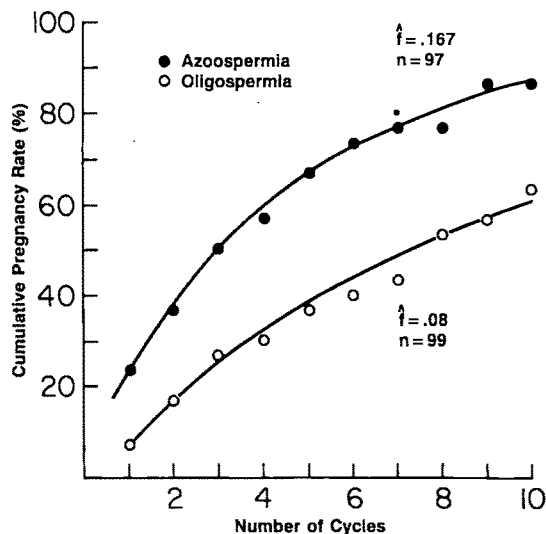


Fig. 2. Cumulative fecundability curves for oligospermia and azoospermia (azoospermia: $\hat{f} = 0.167$, $n = 97$; oligospermia: $\hat{f} = 0.08$, $n = 99$).

Table I. Annual analysis of results of artificial donor insemination

	1980-1981	1981-1982	1982-1983	1983-1984	Total
No. of cycles	139	189	323	352	1003
No. of inseminations per cycle	1.8	1.7	1.8	1.9	
No. of pregnancies	18	27	30	27	102
$\hat{f}$	0.13	0.14	0.093	0.077	0.102
Pregnancies (%)					45.6

Data were analyzed by life-table analysis as described by Cramer et al.¹⁰ The average monthly fecundability, $\hat{f}$ (number of pregnancies per number of cycles), was compared for each treatment group. Corrected cumulative pregnancy rates were also determined. Significant differences were determined with Student's *t* test and χ^2 analysis.

Results

Overall results. A total of 226 patients initiated treatment. One hundred two pregnancies occurred in 1003 treatment cycles for a raw pregnancy rate of 45.6% and a monthly fecundability of 10.2%. No pregnancies occurred after 10 cycles of treatment although 64 further treatment cycles were performed in 14 patients. Analysis was therefore limited to the first 10 cycles. The annual uncorrected results in the artificial insemination with donor sperm program are reported in Table I. The abortion rate was 16.4%. The abortion rate in patients receiving clomiphene therapy was 13.8%. No ectopic pregnancies were reported.

Male factor. Patients with male partners with oligo-

Table II. Effect of severity of male factor on fertility rates

	Oligospermia		Azoospermia	
	All patients	No female factors	All patients	No female factors
Total No. of patients	99	23	97	39
Total No. of cycles	478	58	342	106
Total No. of pregnancies	38	11	57	25
$\hat{f}$	0.08*	0.19	0.17*	0.24
Pregnancies (%)	38.4	47.8	58.7	64.1

*Significant difference by *t* test, $p \leq 0.0001$.

spermia, defined as a detectable number of motile sperm at semen analysis but with severely reduced count or motility, had a significantly lower fecundability rate (Fig. 2) than those with azoospermia ($\hat{f} = 0.079$ versus 0.167, $p \leq 0.0001$). Correction for female fertility factors eliminated the statistical significance although the trend was still apparent (Table II).

No female factors. There were 70 couples in whom

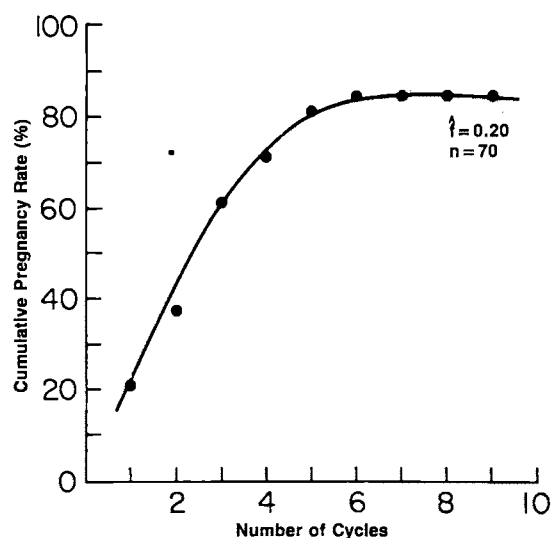


Fig. 3. Cumulative fecundability curve for male factor only (196 cycles, $\hat{f} = 0.20$, $n = 70$).

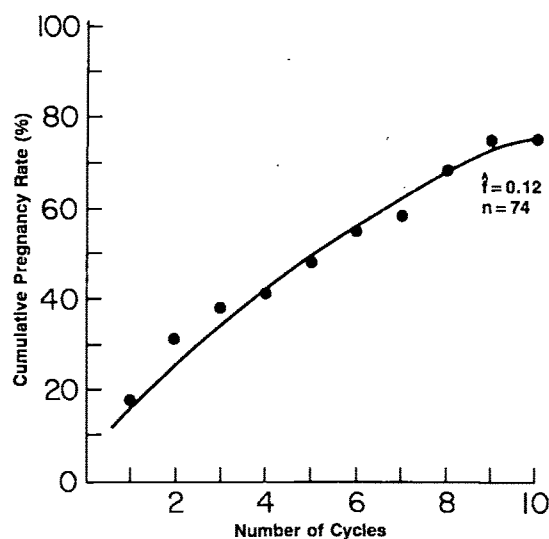


Fig. 4. Cumulative fecundability curve for patients with ovulatory dysfunction (354 cycles, $\hat{f} = 0.12$, $n = 74$).

no female factors were identified before or during artificial insemination with donor sperm. The pregnancy rates in this group, as expected, were the best overall. There were 196 treatment cycles and 38 pregnancies, and the fecundability was 0.20 (Fig. 3).

Ovulatory factor. Patients with anovulation, irregular ovulatory cycles, and luteal phase dysfunction (based on a midluteal progesterone level ≤ 12 mg/ml, a luteal phase < 12 days according to basal body temperature, or abnormality of endometrial biopsy specimen) were treated with clomiphene citrate as described in the Methods section.

The fecundability rate in patients with ovulatory fac-

Table III. Effect of ovulatory dysfunction on pregnancy

	All cycles	All cycles with clomiphene	Cycles with clomiphene and hCG
Total No. of cycles	354	169	108
Total No. of patients	82	67	33
Total No. of pregnancies	41	29	10
$\hat{f}$	0.12	0.17	0.09
Pregnancies (%)	50	43.3	30

RCG = Human chorionic gonadotropin.

Table IV. Effect of fresh versus frozen semen

	Frozen	Fresh and frozen
Total No. of patients	155	73
Total No. of cycles	591	383
Total No. of pregnancies	56	47
$\hat{f}$	0.09	0.12
Pregnancies (%)	36	30

tor infertility was not different from that of the overall population (see Table III and Fig. 4). However, when cycles in which clomiphene was not used were deleted, fecundability rates increased to values similar to those in couples with no female factors.

Endometriosis. Thirty-five patients were identified who had endometriosis, diagnosed at laparoscopy before or during artificial insemination with donor sperm treatment. Nine patients (26%) conceived in 218 cycles. Fecundability rates (0.04) were reduced in this group (Fig. 5). Some patients had ovulatory dysfunction as well.

Fresh versus frozen semen. In the first 3 years of the study patients received three cycles of frozen semen. If they failed to conceive fresh semen was substituted. In the last year frozen semen use was continued to five cycles. Fresh semen was used when available or after five cycles. Inseminations were duly coded as fresh or frozen and numbered by cycle. Results of treatment with fresh and/or frozen semen are reported in Table IV and Fig. 6. The differences in fecundability were not statistically significant.

Comment

The use of donor semen for the therapy of male infertility has been widely reported.^{1-6, 11, 12} Most large programs use sophisticated donor screening including stringent semen quality requirements and genetic screening and are increasingly using microbiologic

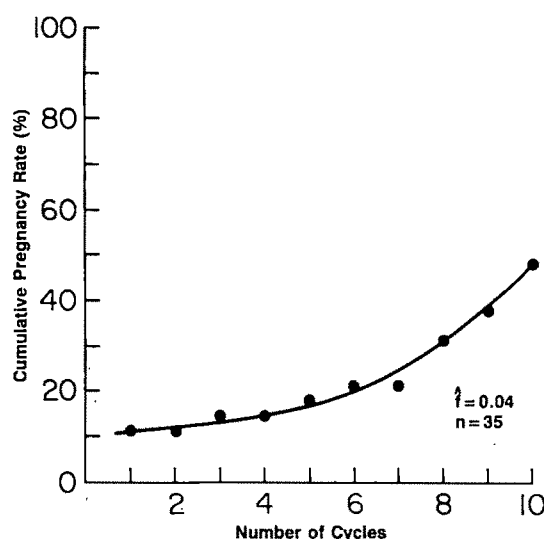


Fig. 5. Cumulative fecundability curve for patients with endometriosis (218 cycles, $\hat{f} = 0.04$, $n = 35$).

screening including testing for hepatitis, gonorrhea, syphilis, and more recently acquired immunodeficiency syndrome. So that the extensive screening will be financially feasible, it has become increasingly common to stockpile multiple samples from these donors in frozen sperm banks allowing for ease in scheduling and reproducibility of samples. Recent evidence suggests that acquired immunodeficiency syndrome can be transmitted by donor sperm, and serial donor testing and storage of samples may be the best method to prevent transmission. Thus results from frozen semen programs will be of increasing significance to patient selection and counseling.

Our results deal with a primarily frozen semen program: Comparison of pregnancy rates within the program suggests a slight but not significant decrease in pregnancy rates with frozen sperm. Our fecundability rate with frozen sperm (0.09) is higher than the 0.05 reported by Richter et al.⁴ but similar to that reported in France³ (0.077 to 0.125), suggesting different donor selection or cryopreservation techniques. Our fresh rates (0.12) are lower than those of many reports^{1, 3-5} (0.15 to 0.20), but at least 52% of our patients had additional infertility factors.

As previously reported,⁵ higher pregnancy rates were noted in patients with azoospermia as opposed to oligospermia. This is almost totally explained when correction is made for female factors, which occur more frequently in this group.

Ovulatory dysfunction was a relatively common female factor (36%) and probably represents a response to the frequency of cycle length variation in these stressed patients. Of interest, pregnancy rates were normal in treated patients.

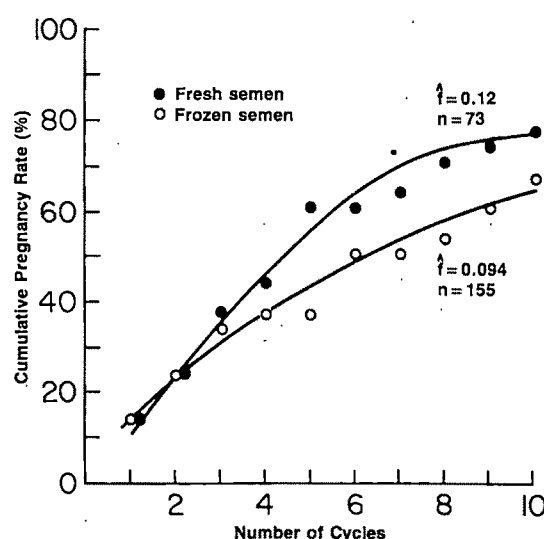


Fig. 6. Cumulative fecundability curves for fresh or frozen semen (fresh: $\hat{f} = 0.12$, $n = 73$; frozen: $\hat{f} = 0.094$, $n = 155$).

Endometriosis, primarily mild, was a significant factor in reduced pregnancy rates, with fecundability in the first six cycles of 0.035. This was improved to 0.073 in the three cycles after danazol therapy.

Tubal and cervical mucus factors were not examined in this study, but in our previous analysis⁶ poor cervical mucus was a factor in reducing pregnancy rates.

The findings suggest acceptable rates with frozen donor semen and acceptable pregnancy rates in female patients with ovulatory dysfunction and treated endometriosis.

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Discussion

DR. R. DON GAMBRELL, Augusta, Georgia. This is an excellent study of an increasingly important infertility factor that will add much to our understanding regarding artificial donor insemination. This report is timely in that the male factor is increasing across the United States and fresh semen cannot be used at present because of the risk of acquired immunodeficiency syndrome. As an aside, I wonder how long it will be before we have to change our time-honored initials for donor insemination, AID, because of the AIDS crisis. DIA does not seem appropriate either since it is too close to DOA. One of the outstanding features of this paper is the emphasis that infertility is not always a single factor and the best results will be achieved with complete evaluation and treatment of all identifiable factors. This group from Chapel Hill has been thorough in their evaluation as evidenced by their good results. My only disagreement in their workup is that I believe timed endometrial biopsies are superior to mid-luteal phase progesterone levels since we are more interested in endometrial tissue response to progesterone rather than serum levels. In treating luteal phase deficiency, we have had better results with progesterone suppositories than with clomiphene citrate and/or human chorionic gonadotropin. In fact, some clomiphene-induced ovulations may not result in pregnancy because of more luteal deficiency and must be supplemented with progesterone suppositories.

The authors are to be congratulated on their results, especially that frozen semen was apparently as effective as fresh semen. The raw pregnancy rate of 45.6% is superior to those of most other reports and certainly better than what we have observed in our clinic, especially with frozen semen. One factor is probably that they have been using 1 ml aliquots of frozen semen where we were using 0.5 ml aliquots. One question is why did the male factor influence pregnancy rates? With oligospermia, the fecundability was 0.08 but with azoospermia it was twice that, at 0.167. Another question for Dr. Hammond is what were the dosages of danazol and for how long were patients treated? They noted that their fecundability doubled from 0.04 to 0.073 after treatment with danazol; however, this was still less than that of most of their other groups. In a study we reported to the American Fertility Society in 1982,¹ the overall pregnancy rate was 91.7% in patients

treated for endometriosis for 6 months with menstrual-suppressing dosages of danazol, that is, 400 to 800 mg, when no other infertility factor were present. With other infertility factors, and oligospermia was a factor in 23.7%, the pregnancy rates were 50.0% after treatment for endometriosis, with no significant differences between the various groups.

Serious genetic disease can be transmitted through donor insemination,² so donor screening must be done as pointed out by the authors. More currently significant is the problem with acquired immunodeficiency syndrome. We recently had to abandon use of fresh donor semen since there is no current method to detect the human T-cell lymphotropic virus type III (HTLV-III) virus in seminal fluid. It has been reported from Australia that a semen donor recipient complaining of symptomless lymphadenopathy may have acquired immunodeficiency syndrome from virally contaminated semen.³ One of four semen donors was seropositive for anti-HTLV-III and three other recipients of semen from this donor were also seropositive for anti-HTLV-III. Seroconversion in patients with acquired immunodeficiency syndrome may not occur for up to 60 days⁴ so even frozen donor semen should be held in quarantine for 60 days before use. This problem with acquired immunodeficiency syndrome makes Dr. Hammond's study very important and timely. Since it may be some time before fresh semen insemination can be resumed, it is reassuring that frozen semen can be effectively used in donor programs with the careful evaluation and treatment outlined by this group. Finally, I would like to ask Dr. Hammond if she has any experience with intrauterine insemination with the use of washed donor semen? This might be a promising new method that could increase the effectiveness even more.

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DR. EDWARD E. WALLACH, Baltimore, Maryland. Dr. Hammond, several years ago Dr. William Beck described patterns for frozen sperm and indicated that despite normal counts and motility to begin with, certain individuals characteristically, when the frozen sperm was thawed, had marked reduction in motility. In the introduction to your article you described the criteria used before cryopreservation of sperm. Did you further select by the characteristics of motility after thawing in these individuals?

DR. GEORGE D. MALKASIAN, Rochester, Minnesota. Dr. Hammond, you described screening your donors for HTLV-III. Are you screening the recipients?

DR. WILLIAM J. LEMAIRE, Miami, Florida. Dr. Hammond, are you now using the rapid urinary luteinizing hormone test and ultrasound to try to time the insemination?

DR. HAMMOND (Closing). I will try to deal with some of these as best that I can. I think the use of endometrial biopsy versus serum progesterone levels in screening for luteal phase defect is a very controversial area that I am afraid I cannot answer today. We prefer to use serum progesterone levels because they are more readily available, they are cheaper, and they are less painful for the patient. We will do endometrial biopsies in our patients after multiple cycles if they fail to conceive. We have had very good luck with Clomid for luteal phase defects and feel that the luteal phase inadequacies reported are probably due to inadequate follicular stimulation, Clomid doses that are too low, and we usually increase our Clomid dose rather than adding progesterone suppositories in our patients.

In response to Dr. Gambrell's questions about the quality of the freezing, we think that perhaps our frozen rates are slightly higher because we use higher numbers in our semen preparations. Our biologic criteria for freezing are very high and are higher than those of any other sperm banks in the country. We also use a specialized freezing technique, which involves a slow freeze and a fairly rapid thaw, that seems to give a higher number of motile sperm. I want to thank Dr. Wallach for reminding me that among our criteria for frozen sperm donors is that 50% of the sperm have to be motile after thawing. We have about a 70% motility requirement before thawing and 50% after freezing. Half of them have to be motile, and there are a large number of men with very good semen analysis, but, as our bank puts it, the sperm of these men cannot be frozen and that is important.

In terms of oligospermia-azoospermia, part of that is corrected if we eliminate patients who have female

factors. It looks like many of the couples with oligospermia also have a high incidence of female factors and perhaps, if the woman had been normal, the oligospermic husband would have been able to produce a pregnancy. So, when we correct our data for female factors, the rates of pregnancy in azoospermic and oligospermic couples improve but are not 100%. One theory is that in oligospermia the sperm are abnormal and may be more likely to incite antisperm antibodies in the female partner. This might also apply to our donor sperm so that we may get reduced pregnancy rates because of sperm antibodies.

In terms of the danazol study, I do not think our analysis was very thorough. We used 600 mg/day for 3 to 6 months, depending on the severity of the disease. Most of these patients have very mild to minimal endometriosis and it may be that their infertility cause is unidentified rather than the endometriosis that we reported here.

Dr. Malkasian's question is a good one. The person in charge of our sperm bank, who is very concerned about the feelings of her donors, was very offended when we wanted to screen the donors and wanted to know why we were not screening the recipients too. We have not been screening recipients either for gonorrhea or for VDRLs or any of these things. We feel that it would just be too expensive.

The last question was Dr. LeMaire's, about how we time our inseminations. There is a lot of interest in the luteinizing hormone kits, the home testing kits, that involve testing urine every day for the luteinizing hormone peaks or doing daily ultrasound examinations in the clinic to pinpoint the time of ovulation. We feel, in normally ovulating women, if we do inseminations every other day until the temperature rises, starting on about the twelfth day of the cycle, that we almost always overlap ovulation. If the cervical mucus is good, sperm survive for 48 to 72 hours in the mucus and these other tests just represent too expensive ancillary treatments to really aid us in improving our pregnancy rates.

Alterations in platelet concentration and aggregation in normal pregnancy and preeclampsia

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Platelets were evaluated in normal pregnant women and in pregnant subjects with mild and severe preeclampsia, nonpregnant control subjects, and pregnant subjects with chronic hypertension. The parameters studied included platelet count, presence of circulating platelet aggregates, and in vitro platelet aggregability by means of a variety of agonists of platelet aggregation. When subjects with a normal pregnancy were compared to nonpregnant controls, they demonstrated a significantly lower platelet count and an increase in circulating platelet aggregates and in vitro hypoaggregability. Significant differences among the groups of pregnant subjects could not be found. These studies suggest the occurrence of platelet activation in pregnancy. This activation causes in vivo platelet aggregation followed by exhaustion of platelets. (AM J OBSTET GYNECOL 1986;155:486-90.)

Key words: Platelets, platelet aggregation, pregnancy, eclampsia, pregnancy-induced hypertension

Pregnancy is accompanied by significant alterations in hemostatic mechanism and its parameters. Studies have indicated overall increase in plasma procoagulant factors, suppression of fibrinolysis, increase in soluble fibrin, and increase in fibrin degradation products.¹⁻⁴ A decrease in platelet number has also been reported to occur.⁵ McKay¹ has suggested that pregnancy is a state of chronic intravascular coagulation, and preeclampsia is apparently the exaggerated state of this phenomenon. The role of platelets in this altered state of hemostasis remains somewhat disputed and unclear. Thrombocytopenia, frequently observed with the onset of pregnancy-induced hypertension, has been considered to represent the consumption of platelets at the microvascular level.⁶ On the other hand, there are also suggestions that platelet involvement may be an important and primary step in the alteration of hemostatic process in some abnormal pregnancies.⁷ The improvement of hemostatic abnormalities in pregnancy-induced hypertension with the use of antiplatelet agent aspirin appears to suggest such a primary role of platelet activation in these pregnancy-induced hemostatic abnormalities.⁸ In view of these possibilities, we studied circulating platelet aggregates and platelet aggregabil-

ity in normal pregnant subjects, in subjects with hypertensive disorders in pregnancy, and also in a large number of nonpregnant subjects as controls.

Material and methods

Four groups were studied. Nonpregnant controls were men and women between the ages of 18 and 35 who had no known medical disorder and who had not taken aspirin, other salicylates, or nonsteroidal antiinflammatory medication for a period of 30 days before study. None of the women were taking estrogen oral contraceptives. We did not control for phase of the menstrual cycle.

Control pregnant subjects were outpatients attending the antenatal clinic or hospitalized patients with either threatened preterm labor or placenta previa. No subject had received medication other than prenatal vitamins for at least 72 hours before sampling. Ten subjects were in early labor at the time of sampling. Subjects with preeclampsia were identified according to the criteria of the Committee for Maternal Welfare of the American College of Obstetricians and Gynecologists and classified as having mild or severe disease according to the committee's definitions. All subjects in this group were inpatients at the time of their studies and eight of these subjects were in early labor at the time of sampling. Subjects with chronic hypertension during pregnancy were derived from both inpatient and outpatient populations, and none of them were in labor at the time of the study. Following the obtaining of written informed consent, samples were obtained from nonoccluded forearm vessels via a 19-gauge needle attached to silicone rubber tubing. Fifty milliliters

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of blood were collected into a plastic syringe containing a final volume of 10% (vol/vol) of 3.8% sodium citrate. The circulating platelet aggregation ratio was determined with use of the method of Wu and Hoak.⁹ In this technique, freshly drawn blood is anticoagulated with ethylenediaminetetraacetate or ethylenediaminetetraacetate and formalin. Platelet-rich plasma is prepared by centrifugation, and platelet aggregates that have been fixed by formalin are sedimented out. Thus the formalin-ethylenediaminetetraacetate-platelet-rich plasma/ethylenediaminetetraacetate-platelet-rich plasma platelet count ratio is decreased if platelet aggregates are present in the freshly drawn blood.

Platelet counts were determined via a Coulter Thrombocounter-C Model. Platelet-rich plasma was prepared by centrifugation (500 g for 5 minutes) and stored in plastic containers at room temperature.

Aggregation was measured photometrically in 0.4 ml volumes of platelet-rich plasma containing 300,000 cells/mm³ by means of a BioData Model 2A aggregometer with use of standard methods. Maximal aggregation was measured for each sample in response to the addition of 0.1 ml of adenosine diphosphate (2.5×10^{-5}), epinephrine (1×10^{-4} mol/L), collagen (1.9 mg/ml), and arachidonic acid (5 mg/ml). All studies contained at least one subject from the nonpregnant and normal pregnant control groups.

Results

Platelet aggregation in response to different agonists (adenosine diphosphate, collagen, epinephrine, and arachidonic acid) was studied in 30 pregnant patients. This was compared with the studies in 20 nonpregnant individuals used as controls. Circulating platelet aggregates were also studied in these two groups. Results are depicted in Table I. When compared to controls, the pregnant patients demonstrated a statistically significant lower circulating platelet aggregate ratio (indicating circulating aggregates), and the threshold of their platelet aggregation was reduced with each of the agents tested. There was no apparent difference in time required for maximal aggregation, and slopes were not analyzed. The platelet count in pregnant subjects was lower, and this lowering was also statistically significant when compared to nonpregnant controls. The pregnant subjects were younger (23.8 ± 2.1 years), but this difference was not significant.

Platelet aggregation and circulating platelet ratios were also determined for pregnant subjects with mild or severe preeclampsia or chronic hypertension. Normal pregnant subjects served as controls for these studies. The clinical parameters and number of subjects studied in each group are shown on Table II. The subjects with chronic hypertension were somewhat

Table I. Platelet function in normal pregnant women and nonpregnant controls (% aggregation, mean $\pm$ SEM)

	Nonpregnant (n = 20)	Pregnant (n = 30)	p*
Age (yr)	27.8 $\pm$ 2.1	23.8 $\pm$ 1.0	NS
Platelet count ($\times 10^3$ /ml)	354 $\pm$ 16	285 $\pm$ 18	<0.01
Platelet aggregate ratio	0.88 $\pm$ 0.05	0.74 $\pm$ 0.03	<0.05
Adenosine diphosphate	71.9 $\pm$ 5.7	54.9 $\pm$ 5.3	<0.05
Collagen	84.5 $\pm$ 3.7	56.8 $\pm$ 5.0	<0.01
Epinephrine	79.3 $\pm$ 6.5	55.1 $\pm$ 5.9	<0.01
Arachidonic acid	87.2 $\pm$ 3.2	61.4 $\pm$ 5.7	<0.01

*Student's unpaired *t* test, two-tailed.

older and were studied at an earlier gestation age. The mild preeclampsia and severe preeclampsia groups contained higher percentages of nulliparous subjects but were of similar ages and gestational ages when compared to the controls.

Comparisons between subjects with hypertensive disorders and normal pregnant women may be seen in Table III. When compared to normal controls and women with chronic hypertension, subjects with preeclampsia showed a trend toward lower platelet aggregation ratios and decreased response to aggregating agents, but these differences failed to achieve statistical significance.

Comment

Studies presented in this report indicate that normal or abnormal pregnancy is accompanied by significant alteration of platelet function. These results clearly show that the platelets in pregnant subjects are hypoaggregable when examined in the presence of a variety of agonists including adenosine diphosphate, epinephrine, collagen, and arachidonic acid. This suggests the possibility that platelets are being activated in vivo. The possibility of in vivo activation is supported by the finding that pregnant subjects also have a significantly higher number of circulating platelet aggregates when compared to nonpregnant controls.

These findings therefore suggest the possibility that pregnancy is associated with the activation of platelets, which leads to their in vivo clumping and aggregation. The development of circulating platelet aggregates then lead to their removal by the reticuloendothelial system, resulting in a reduction in platelet concentration. Furthermore, during the process of in vivo activation and aggregation, these platelets become partially exhausted. The exhaustion so produced is depicted by the phenomenon of hypoaggregation of these platelets when examined in the in vitro system of platelet aggregation.

The hypothesis presented here regarding the sequence of events and changes in platelets during preg-

Table II. Clinical parameters in pregnant subjects (mean $\pm$ SEM)

	Normal (n = 30)	Mild pregnancy-induced hypertension (n = 23)	Severe pregnancy-induced hypertension (n = 11)	Chronic hypertension (n = 16)
Age (yr)	23.8 $\pm$ 1.0	21.5 $\pm$ 0.9	21.3 $\pm$ 1.4	26.9 $\pm$ 1.4
Gestational age (wk)	34.6 $\pm$ 0.7	36.5 $\pm$ 0.7	36.6 $\pm$ 1.1	30.4 $\pm$ 2.3
Nulliparous (%)	62	80	91	57
Systolic blood pressure (mm Hg)	—	150 $\pm$ 2	172 $\pm$ 3	152 $\pm$ 2
Diastolic blood pressure (mm Hg)	—	97 $\pm$ 2	108 $\pm$ 2	98 $\pm$ 2

Table III. Platelet function in normal pregnant women and women with hypertensive disorders of pregnancy (% aggregation, mean $\pm$ SEM)

	Normal	Mild pregnancy-induced hypertension	Severe pregnancy-induced hypertension	Chronic hypertension
Platelet count ($\times 10^3$ /ml)	285 $\pm$ 18	263 $\pm$ 16	267 $\pm$ 29	319 $\pm$ 29
Platelet aggregate ratio	0.74 $\pm$ 0.03	0.68 $\pm$ 0.07	0.68 $\pm$ 0.07	0.70 $\pm$ 0.05
Adenosine diphosphate	54.9 $\pm$ 5.3	55.3 $\pm$ 7.3	51.8 $\pm$ 8.6	58.5 $\pm$ 8.9
Collagen	56.8 $\pm$ 5.0	57.7 $\pm$ 6.4	47.5 $\pm$ 6.4	68.0 $\pm$ 8.0
Epinephrine	55.1 $\pm$ 5.9	39.3 $\pm$ 7.2	43.8 $\pm$ 8.2	62.3 $\pm$ 7.2
Arachidonic acid	61.4 $\pm$ 5.7	62.1 $\pm$ 6.7	57.5 $\pm$ 9.6	61.6 $\pm$ 7.3

nancy appears to be strengthened by some earlier studies. Whigham et al.⁷ studied the platelet function in preeclampsia and demonstrated that women with severe preeclampsia had raised plasma adenine nucleotide levels and lowered platelet 5-hydroxytryptamine.

Platelet intracellular organelles are rich sources of adenosine diphosphate and 5-hydroxytryptamine. It can be considered that raised adenine nucleotide in the plasma of these patients is due to the release phenomenon following activation of these platelets. The depleted level of 5-hydroxytryptamine in platelets also supports the release and loss of 5-hydroxytryptamine and therefore exhaustion of platelets in circulation of pregnant subjects. Socol et al.¹⁰ studied platelets in preeclampsia and demonstrated that plasma β -thromboglobulin levels were high in third-trimester patients hospitalized with preeclampsia. β -Thromboglobulin is a platelet-specific protein that is released when platelets are aggregated.

An active role for platelets in the pregnancy-induced alteration of hemostasis remains disputed. Relatively few normal pregnant women have been studied. The majority of the work has emphasized the role of intravascular coagulation. Severe preeclampsia has been reported to be associated with features of intravascular coagulation. This has included fibrin deposition in tissues, increased levels of fibrin degradation products, together with thrombocytopenia. Redman¹¹ has suggested that coagulation problems in pregnancy are primarily associated with overactivity of intrinsic clotting system. The role of platelets in the pathogenesis of altered hemostasis in pregnancy may not have been fully explored. It has been suggested that platelets are involved passively in the process of disseminated intra-

vascular coagulation or are sequestered and trapped passively in microcirculation of organs such as placenta, kidneys, and liver.¹ It is possible, however, that platelets do play an important and active role in the pathogenesis of the hemostatic abnormalities.

Our finding of in vivo circulating platelet aggregates and in vitro hypoaggregation in pregnancy-induced hypertensive states as well as in normal pregnancy appears to support the concept that platelets are intimately involved in these states and are not just being consumed during the process of an ongoing situation similar to disseminated intravascular coagulation.

Various possibilities can be considered regarding the mechanism of activation of platelets in pregnancy. Some of these could be activation by immune complexes, by endothelial cell injury in the systemic circulation, or by contact with trophoblastic cells in placental circulation.¹² Recently, enhanced reactivity of the platelet thromboxane pathway has been shown to occur in normotensive and hypertensive pregnancies.¹³ Furthermore, reduced umbilical and placental prostacyclin has been reported in severe preeclampsia.¹⁴ Prostacyclin activity has also been found to be significantly reduced in amniotic fluid in preeclampsia.¹⁵ Prostacyclin is an important hormone that exerts strong inhibition of platelet aggregation. It has been suggested that there is continuous availability of this hormone from blood vessels,¹⁶ which keeps circulating platelets in a dispersed and disaggregated form. It could be inferred from these findings that deprivation of this prostacyclin in pregnancy-induced hypertension can make the circulating platelets even more vulnerable to aggregation and activation in retroplacental space. The removal of aggregated platelets might be responsible for the

thrombocytopenia often observed in pregnancy-induced hypertension. The primary role of platelet activation appears to gain more strength by the recent observation that the use of the antiplatelet agent aspirin has produced amelioration of the clinical picture of preeclampsia and caused a rise in platelet count in thrombocytopenic preeclamptic patients.

In summary, this study demonstrated an increase in circulating platelet aggregates and a reduction in platelet aggregability in normal pregnant women and in women with the hypertensive disorders of pregnancy. These findings appear to attribute an important and active role to the platelets in the hemostatic alterations in both normal and abnormal pregnancy and should focus attention on the activation of platelets in the retroplacental space as well as the systemic circulation.

We would like to acknowledge Ms. Anita Bruce of the Division of Hematology for her technical assistance on this project.

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Discussion

DR. ROBERT C. CEFALO, Chapel Hill, North Carolina. This is an interesting study, which clearly demonstrates a difference in maximum platelet aggregation between nonpregnant and pregnant patients. It extends previous observations that indicate a state of chronic platelet activation and shortened platelet life span during pregnancy. However, within pregnant subgroups of patients, significant differences in platelet function were not demonstrated. The inability to demonstrate any difference among the subgroup of pregnant patients studied would suggest that platelet dysfunction does not play a primary role in the pathogenesis of pregnancy-induced hypertension and its associated hemostatic abnormalities. Although the authors rely on anecdotal reports of clinical improvement of pregnancy-induced hypertension in patients treated with antiprostaglandin compounds such as aspirin, there are probably an equal number of reports that document no clinical improvement or deterioration of patients treated in this fashion.

Dr. O'Brien and his associates have chosen to study two parameters of platelet physiology, circulating platelet aggregates and platelet function in vitro. They are to be commended for their inclusion of a large number of subjects in their groups, especially normal pregnant women, which is a significant improvement over previous studies in which only small numbers of subjects were studied. Their results demonstrate quite convincingly that platelets of pregnant women are considerably less responsive to standard aggregating agents and demonstrate evidence of circulating clumps of platelets.

The following statements and questions are presented for Dr. O'Brien:

- One might argue that the random use of nonpregnant women is inappropriate since platelet function can vary with the menstrual cycle.
- Were any of the women in the nonpregnant control groups ingesting oral contraceptives?
- Were all of the patients in labor at the time of venipuncture or are the pregnant subgroups a mixture of laboring and nonlaboring patients?
- What standard of platelet count was used to perform the aggregation studies—200,000 or 300,000 cells/mm³? Did the investigators analyze the rate of platelet aggregation (based on the slope) to evaluate differences among the groups? Were there any significant differences in the time required for maximum platelet aggregation among the different groups?

In summary, the study adds considerably to our knowledge of physiology in pregnancy and presents an interesting hypothesis for the fall in platelet count during pregnancy noted by many authors. It fails, however,

to provide any new clues to the pathogenesis of preeclampsia. Studies soon to be reported from our laboratory at the University of North Carolina at Chapel Hill substantiate the rapid degradation of prostacyclin in patients with preeclampsia. Prostacyclin is an inhibitor of platelet aggregation, and there appears to be decreased production in pregnancy-induced hypertension. Our studies indicated a significant difference in platelet aggregation in nonpregnant women, in women with an uncomplicated pregnancy, and in preeclamptic women in relationship to prostacyclin action. There may be an as yet unknown plasma factor that may explain our findings and those of Dr. O'Brien reported in his study.

DR. O'BRIEN (Closing). I am glad that our studies at different sites are coming to the same basic conclusion. I agree with Dr. Cefalo's major criticism of the study, namely that although we set out to look for this mysterious difference in platelet function that has been reported to occur in patients with pregnancy-induced hypertension, we did not see any dramatic change. Perhaps the major beneficial point of the paper is that I think we have now demonstrated that anybody who does attempt to show a change in platelet function in patients with pregnancy-induced hypertension better have conclusively shown that they are different from normal third-trimester pregnancy controls. We did not look at the phases of the menstrual cycle in our nonpregnant subjects although we could go back and do that. If one looks at the literature, there are only slight

changes across the phases of the menstrual cycle which are nowhere near as dramatic as the type of changes that we saw between controls and pregnant patients. None of the patients were on oral contraceptives at the time they were allowed to be subjects. I did not include that, but that was part of our criteria. In terms of whether or not the patients were in labor, none of the patients had uterine contractions. There were patients who were in preterm labor who were in the control group, but none of the patients were having contractions at the time. I should mention that none of the patients we used, with either mild or severe pregnancy-induced hypertension, were on magnesium sulfate at the time the platelets were sampled because certainly in vitro magnesium levels can alter platelet function and we did not want that to interfere with our results. We did look at the slope of aggregation as well as the time to maximal aggregation; there did not appear to be a difference across the groups, and we did not analyze it in any greater detail than that. We felt that with the large numbers that we had in our study the maximum amount of platelet aggregation was the most specific point that we could use.

Finally, I agree with Dr. Cefalo that this paper may demonstrate more than anything else that pregnancy alters the milieu in which pregnancy-induced hypertension may work its effects on platelet function. It may not have a specific effect directly on platelets in terms of etiology, but perhaps it is the milieu that makes the pregnant woman so susceptible to this disease.

Brief reports with panel discussion

I. Arteriographic management of uterine arteriovenous fistula

Ronald L. Brown, M.D., A. Van Moore, M.D., and Alexander R. Smythe, M.D.

Columbia, South Carolina, and Charlotte, North Carolina

A 21-year-old woman had an arteriovenous fistula of the uterus diagnosed by arteriography after several episodes of profuse uterine bleeding. Arteriographic embolization of the uterine vessels was performed twice with the resumption of normal menses after the second procedure. 15-Methyl-prostaglandin $F_{2\alpha}$ was used effectively for short-term control of hemorrhage. (AM J OBSTET GYNECOL 1986;155:491-3.)

Key words: Uterine arteriovenous fistula

Uterine arteriovenous fistula is a rare entity that can result in life-threatening hemorrhage. It has been described as associated with congenital abnormality, trophoblastic disease, and trauma.¹ Arteriography has been used previously to identify fistulas,² but to our knowledge no one has occluded a fistula without performing a major operation.

Case presentation

The patient, a 21-year-old white woman, gravida 2, para 0, therapeutic abortions 2, underwent first-trimester dilatation and evacuation, performed in May, 1983, and second-trimester dilatation and evacuation, performed at 16 weeks with some difficulty, in August, 1983. Normal, although somewhat heavier menstrual periods followed until February, 1984. She was amenorrheic for 2 months and was given Provera, 10 mg, for 5 days after a negative urine pregnancy test. She then had a withdrawal period in April, 1984, with severe hemorrhage, requiring curettage, and a blood transfusion of 2 units. In May, 1984, she had a similar episode and was treated with intravenous estrogen, and another curettage was done with the bleeding finally stopping. She then started to use oral contraceptives.

In June, 1984, she was evaluated with hysteroscopy, which revealed atrophic endometrium with no other anatomic abnormalities. The following day a pelvic arteriogram revealed a large arteriovenous malformation of the uterus. Embolization of the right uterine artery resulted with placement of a Dacron and stainless steel coil in the proximal uterine artery. The left uterine artery was unable to be catheterized selec-

tively, and two coils were placed just proximal to the left uterine artery. Postembolization arteriogram revealed greatly decreased blood flow to the area of the fistula. The patient was then placed on a regimen of continuous oral contraception for 4 months.

In October, 1984, she again had profuse uterine bleeding. She was hospitalized and again transfused with 2 units of packed red blood cells and treated with parenteral estrogen and methylergonovine. The bleeding subsided, and she restarted oral contraception. In December, 1984, she was again hospitalized with profuse bleeding and treated with the previous regimen and additionally with 15-methyl-prostaglandin $F_{2\alpha}$, which promptly stopped the bleeding. During the next 2 months, the patient had two further episodes of bleeding, both of which were abruptly stopped with prostaglandin administration. She required no further blood transfusion.

In February, 1985, the patient had repeat arteriography performed, revealing the previously noted arteriovenous fistula. Embolization of the uterine arteries was carried out through a left axillary artery approach. The procedure was complicated by the presence of the previously placed coils. The coil in the right uterine artery was circumvented and another coil and Gelfoam particles were used to occlude the right uterine artery adjacent to the uterus. More difficulty was encountered on the left side and the uterine artery was occluded proximal to the previously placed coil with two other coils and a large Gelfoam plug. Postoperative arteriogram revealed excellent blockage of the right uterine artery and proximal uterine artery on the left side. Ultrasound and Doppler studies performed 1 week later revealed greatly reduced blood flow to the area of the fistula.

She had one further bleeding episode 3 weeks later, which stopped with administration of prostaglandin $F_{2\alpha}$. In April, 1985, repeat arteriography revealed no evidence of arteriovenous fistula. Subsequently, the patient has been maintained on a regimen of cyclic low-dose oral contraceptives and has had normal menses.

Comment

Profuse uterine bleeding in a woman with a similar history should alert one to the possibility of arteriovenous fistula. In a woman who desires future childbearing, arteriography may provide a diagnostic and treatment modality that could obviate hysterectomy. For interim bleeding episodes, the use of 15-methyl prostaglandin $F_{2\alpha}$ may offer an effective means of hemostasis.

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Discussion

DR. GEORGE D. MALKASIAN, Rochester, Minnesota. There are few cases of uterine arteriovenous fistula reported in the

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literature and few anecdotal experiences. Most occur in women who have had multiple pregnancies and in women who have a history of some kind of trauma to the uterus, such as multiple curettages. There is a recent case report of a fistula occurring when a Lippes loop had penetrated the uterine wall to 7 or 8 mm. Several cases have been reported after regression of trophoblastic disease. What we heard today adds to our knowledge. The authors are to be commended for pursuing the embolization techniques. This is something we all have to consider as we deal with these problems. The use of prostaglandins for control of bleeding is most appropriate and something we should all keep in mind. Fortunately, the patient was not asthmatic or hypertensive, which would have precluded the use of prostaglandins. There are side effects with the use of prostaglandins. We have seen three cases of fistulas in recent years. Two followed trophoblastic disease that was treated with chemotherapeutic agents. All have been treated by embolization and there have been no subsequent pregnancies in these individuals. I understand that in one case reported at our meeting last year the patient did have a subsequent pregnancy and I think there is one other report in the Scandinavian literature. How have you counseled your patient regarding subsequent pregnancies? Would it be appropriate to check her again with arteriograms before pregnancy is considered?

DR. BROWN. The patient does indeed need to be evaluated. We will perform Doppler studies, ultrasound evaluation, and magnetic resonance scanning before she attempts pregnancy. Of course, during pregnancy, we will monitor her for the possibility of intrauterine growth retardation.

DR. MALKASIAN. I have one other comment in regard to the reference to ultrasound studies. We did examine one of these patients that I referred to with ultrasound, and our ultrasonographers can actually be more specific than I can be, in that they describe larger cystic areas as associated with arteriovenous fistulas as opposed to the smaller cystic areas one might see with hemangiomas. If there is an arteriovenous fistula, the walls of the cyst on ultrasound are supposed to be thinner. However, I must admit that I cannot distinguish the thin from the thick walls in the two, but they point them out faithfully for comparison. I think ultrasound is a good tool. Initially, if an arteriovenous fistula is suspected and cystic areas are seen in the uterine wall or in the adnexal areas, then arteriographic studies can be performed.

DR. EDWARD E. WALLACH, Baltimore, Maryland. I am going to take the endocrinologic view of this rather than the surgical view. This case is very fascinating and I think it represents the type of case that none of us wants to see and we lie awake at night worrying about. First, I am interested in the mechanism of menstrual bleeding and what we have called dysfunctional bleeding or also irregular bleeding that may be ovulatory. I feel uncomfortable in attributing what we see with this patient to an arteriovenous fistula. I assume that the fistula is thought to have occurred traumatically but I am not sure how one could effect a traumatic arteriovenous fistula in the uterine vessel by a curettage. Even if it were possible, I have difficulty determining how this would give rise to abnormal bleeding. Second, I also wonder about whether the bleeding that was reported here was a result of an atrophic endometrium. We know that the hemostatic mechanism responsible for cessation of bleeding at the end of each cycle is based on adequacy of pseudodecidual formation and also on control of the vasculature of the spiral arteries in the endometrium. The fact that the patient had atrophic endometrium makes me concerned that the bleeding may not have been a result of the fistula but a result of bleeding from an atrophic endometrium. Certainly

the use of prostaglandin, with its effect on myometrium, could explain the control of the bleeding that you experienced on two or three occasions using a prostaglandin analogue. There is a very recent article in the *AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY*, from Tulchinsky's group in Boston, by Giannopoulos et al.¹ who demonstrated that there are prostaglandin E and F receptors in the myometrium in the pregnant and nonpregnant state and that there is indeed a cyclic variation of the concentration of these receptors. Thus there is good reason to suspect that the prostaglandin derivative is controlling the bleeding. I would not have expected Methergine to control bleeding in the nonpregnant myometrium. I would be somewhat concerned about the use of oral contraceptives in a woman who has artificial embolization of the vessels as to the integrity of the endothelium and the possibility for other thromboembolic phenomena during the use of oral contraceptives. I also wondered about the pregnancy potential in this patient, more on the basis of the atrophic endometrium than on the basis of embolization of a malformation or a fistula. I admire you for your zeal in preserving the uterus of this patient. Obviously, the reason is because she is young and her only pregnancies have ended in voluntary terminations. I would look at a subsequent pregnancy with a great deal of trepidation.

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DR. PAUL B. UNDERWOOD, Charlottesville, Virginia. I have never seen one of these cases either. They certainly are very rare. In reviewing the literature, I found 14 cases that had been reported. All of them were single cases, the oldest going back to 1898. One of the members of this society, Dr. George Williams, reported the case in 1954. It is worth considering how these patients present because, when one reviews all of the cases individually, they present very similarly to this patient except that she is far younger than any that has been reported. The youngest that I could find reported was 34 years of age. Several were postmenopausal women, and several of them had had multiple pregnancies. One of them had had more than 10 children. All had had curettage and all of them had had trauma. So, certainly, when you think of an arteriovenous fistula, you think of either a congenital or a traumatic cause. However, not all of these tumors, which I guess you would call them, are associated with trauma, at least by history. They have several names—arteriovenous fistula, as reported, arteriovenous malformation, and the curious term cirroid aneurysms of the uterus. In the cirroid aneurysm, cirroid refers to varicosities. The patients present with a uterus that is two to three times normal size and tender to palpation. They have profuse hemorrhage, as in this case. The hemorrhage, I think, would be too much for atrophic endometrium because, when we review these cases, most of them refer to bleeding amounting to several units of blood in a matter of a few hours. This patient required 6 units of blood transfusion, which is somewhat unc customary. Uterine packing may have to be used, but this is certainly a rare indication for it. In these cases, since these are open venous sinuses that are bleeding, or perhaps arteriovenous sinuses, the packing may be necessary. All of these cases refer to the fact that curettage is extremely dangerous because some other areas may be opened up. These

can usually be suspected by the fact that the uterus actually has a thrill. When one feels these enormously dilated arteries and veins, a pulsating uterus or a thrill to the uterus can be palpated, and several authors referred to the fact that if one listened with a stethoscope a bruit could be heard over the lower abdomen. The thing that bothers me in this particular case, certainly just from reading the others, is that I could find no other case that was treated in this manner. All the others that I was aware of were treated by hysterectomy and removal of the arterial fistula. I think this is an excellent way to treat this patient and I congratulate the authors that they did choose to do so. What worries me, and this is purely a personal worry, is that, since these are dilated vessels, you have stopped or occluded the main blood supply to the uterus, that being both uterine arteries; however, collateral circulation is obviously going to develop and continue to develop. Will this condition recur with time because of the anastomosis to the ovarian arteries? I have no idea. It certainly is a very interesting case, a very rare case, and I hope I never see one.

DR. JAMES M. INGRAM, Tampa, Florida. I would like to add one patient that is not in the 14 cases. This is some work being done at the New York University, originating in the Department of Neurosurgery. There they have been using Krazy Glue (ethyl-cyanoacrylate) to embolize neoplasia and arteriovenous fistulas of the brain. They obtained special permission to use Krazy Glue in a patient that was identical to this one, except one year older, with very good results. This case report has been submitted for publication.

DR. WALLACH. I would still hope that Dr. Brown can explain the pathogenesis of the irregular bleeding at the time of an anticipated menstrual flow, or withdrawal flow, as a result of a fistula between the uterine artery and vein.

DR. BROWN. We assumed that the fistula was covered over by endometrium. In fact, the use of continuous high-dose estrogen oral contraceptives was an attempt to produce a thicker lining over the fistula. When this lining started breaking down, then the "floodgates" would break and the fistula would open up.

DR. INGRAM. Just one comment—when you look at the uterus in some of these pictures (photographs deleted), several of you have even commented that within the myometrial wall there were vessels you could put your finger in. So you are talking about very large vessels within the myometrial wall.

DR. MALKASIAN. In the review of the literature, did anyone do a more extensive workup for congenital arteriovenous malformations? If you think about midline malformations, these patients have similar types of bleeding problems and I wonder if it isn't worth doing a more extensive workup.

DR. INGRAM. I would like to know, if this patient happens to get pregnant, how you plan to deliver—vaginally or by cesarean section.

DR. MALKASIAN. There is one case report from the Scandinavian literature that is very similar to this. The patient was delivered vaginally and at the time of the article was pregnant with her second pregnancy. She had no evidence of intra-uterine growth retardation during her first pregnancy and was delivered vaginally of a normal term infant. So, hopefully, we will get the same result. I think we should give all due respect to another member of this organization who did report a very similar case, Dr. David Chapman, at the South Atlantic meeting either 3 or 4 years ago. He reported a case handled in a manner almost identical to that of Dr. Brown. He then reported, just last year, that this same particular patient had been pregnant and was delivered uneventfully. So there was another case since 1972. We recently, within the last year, have

had a similar case. After an abortion, a demonstrable arteriovenous fistula was corrected by the same technique of arteriography. The interesting thing about it is the profuse bleeding that occurs at the time, whether it is menses or other vaginal bleeding, requiring up to 6 to 8 units of blood. This patient has been lost to follow-up.

II. Glycosylated serum protein level as a screening and diagnostic test for gestational diabetes mellitus

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Glycosylated serum protein assay was examined as an alternative to standard glucose screening and glucose tolerance testing. In a comparison of two groups of gravid women having abnormal 1-hour 50 gm glucose screening tests, there was no difference in glycosylated protein level in the group with abnormal glucose tolerance test results ($9.4\% \pm 2.0\%$, mean $\pm$ SD; $n = 8$) versus normal results ($9.2\% \pm 1.07\%$, mean $\pm$ SD; $n = 11$). Furthermore, correlation of glycosylated serum protein level with glucose screening test results was poor ($r = 0.185$, $p = 0.23$, $n = 17$). Glycosylated serum protein assay is not useful in detecting mild metabolic aberrations associated with gestational diabetes. (AM J OBSTET GYNECOL 1986;155:493-6.)

Key words: Gestational diabetes, glycosylated serum protein, glucose screening test, glucose tolerance test

Glucose tolerance testing is an effective but cumbersome method of diagnosing gestational diabetes. It can, however, fail to identify the pregnancy at risk¹ and is a time-consuming hardship to patients. False tests can result from inadequate patient preparation and coincident illness. Glucose screening tests share these problems. Glycosylated blood substances, including hemoglobin and protein, correlate with integrated blood glucose levels over the time of their existence and have been evaluated as a means not only of evaluating diabetic control,^{2,3} but of identifying diabetes.⁴⁻⁶ This study was designed to evaluate glycosylated serum protein as both a diagnostic and a screening method for detecting gestational diabetes.

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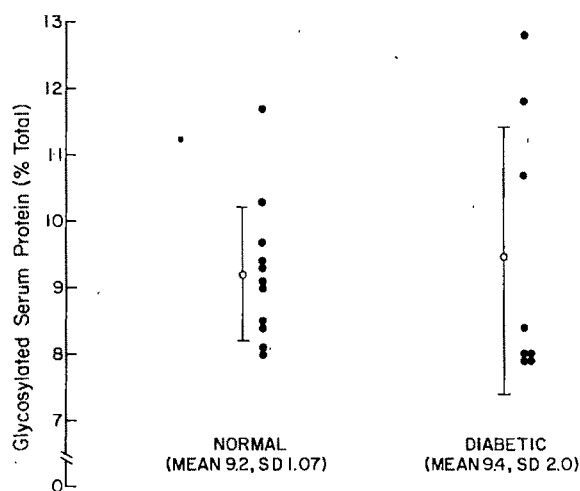


Fig. 1. Glycosylated serum protein testing in 11 normal and eight diabetic pregnant women ($p = 0.79$).

Material and methods

A set of pregnant women with one or more risk factors for gestational diabetes mellitus and with an abnormal glucose challenge test result were randomly selected for study. Risk factors included either family history of diabetes, body weight $>25\%$ above ideal, history of repeated pregnancy loss or unexplained stillbirth, previous delivery of an infant >4000 gm, or previous delivery of an unexplained anomalous infant.

Glucose challenge was performed at a random time while the patient was in good health and normally ambulatory during the second and third trimesters. Serum glucose levels of ≥ 135 mg/dl 1 hour following a 50 gm glucose load were considered abnormal.⁷

All patients in the group had a glucose tolerance test following the abnormal challenge result. These tests were performed while the patient was well and at normal activity. Patients were instructed to consume diets containing at least 150 gm of carbohydrate per day for 3 days before the test and to fast during the night before testing. Testing was done in the morning. Following a fasting determination, serum glucose was measured at 1, 2, and 3 hours after a 100 gm oral glucose load. Gestational diabetes was diagnosed in women with two or more serum glucose test results above the following levels: 105 mg/dl at fasting; 190 mg/dl 1 hour later; 165 mg/dl at 2 hours; and 145 mg/dl at 3 hours.⁸ Several days after the abnormal screen, glycosylated serum protein determinations were made from samples obtained at the time of the glucose tolerance test.

Serum or plasma glucose levels were measured by means of glucose hexokinase enzymatic assay of the end-point reaction. Glycosylated serum protein was determined by column separation of glycosylated from nonglycosylated protein (GLYC-AFFIN System, Isolab, Akron, Ohio) and by spectrophotometric analysis. Ranges of "normal" for normal patients and nonpregnant diabetics, as a percent of total, were: normal patients, mean = 10.7, range = 8.0 to 12.9; diabetic patients, mean = 15.3, range = 10.0 to 23.9. Statistical

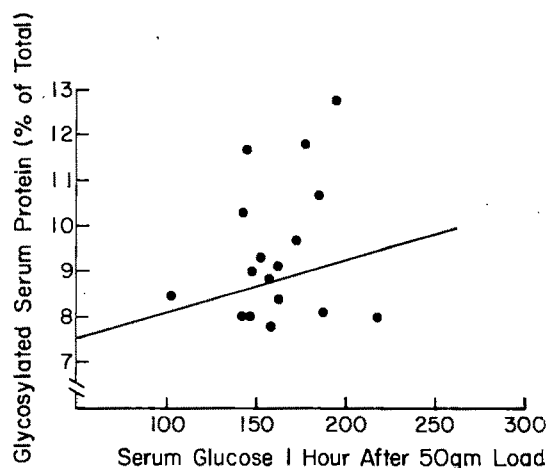


Fig. 2. The correlation of glycosylated serum protein testing and 1-hour glucose screening ($r = 0.185$, $p = 0.23$, $n = 17$).

evaluation of the data was by Student's t test and regression analysis.

Results

Of 19 patients with abnormal 1-hour glucose challenge tests, eight had abnormal glucose tolerance test results and were classified as gestational diabetics. The level of glycosylated protein for this group ($9.4\% \pm 2.0\%$, mean $\pm$ SD) was not different from that of the remaining 11 patients with normal glucose tolerance tests ($9.2\% \pm 1.07$, $p = 0.79$, $t = 0.27$) (Fig. 1).

Glycosylated serum protein level was compared to serum glucose after glucose challenge (1-hour, 50 gm) in 17 of the women. As shown in Fig. 2, correlation was poor ($r = 0.185$, $p = 0.24$, $n = 17$) with a regression equation of $y = 7.5 + 0.11x$.

Comment

Glycosylated substances can be useful adjuncts to serum glucose measurements in diabetic patients. The relative simplicity of a single blood test that does not require patient preparation is attractive. Glycosylated hemoglobin testing has already been found useful to assess glucose control over the protracted period of 6 to 8 weeks.⁹ Glycosylated serum protein testing offers an advantage because the more rapid turnover of serum proteins reflects glucose control during a more confined period of 2 to 3 weeks.¹⁰ Both tests have raised the possibility of a method superior to the glucose tolerance test for diagnosis of diabetes.

The detection of gestational glucose intolerance is of undisputed importance.¹¹ While overt diabetes presents difficult challenges to management skills, gestational diabetes offers primarily a problem in diagnosis. Glucose tolerance tests are difficult and time-consuming for the patient and not always accurate.¹ Screening tests used to identify the patient requiring the more cumbersome glucose tolerance test share these disadvantages.⁸

A correlation between ambient blood glucose, as

measured by glycosylated hemoglobin or protein, and actual blood sugar determinations would suggest that these tests might efficiently reveal glucose intolerance. Depending on degree of blood glucose elevation, they do. But in a 1978 study of glucose tolerance tests in nonpregnant patients, only half of the subjects diagnosed as having mild (chemical) diabetes had an elevated glycosylated hemoglobin level.⁴ A correlation between glycosylated hemoglobin and blood sugar levels exists in pregnant women¹² but is too weak to allow this test to substitute for glucose tolerance testing in patients with the relatively small aberrations of blood glucose level typical of gestational diabetes.^{5,6}

Glycosylated serum protein levels bear a significant correlation with serum glucose levels in nonpregnant¹⁰ and pregnant diabetic patients.¹² A single previous attempt to apply this index of glucose level to the diagnosis of gestational diabetes demonstrated a significant mean difference in protein between diabetic and normal subjects.⁶ However, the degree of overlap between 17 normal and eight diabetic women in that study was sufficient to preclude use of the test to distinguish individuals as being diabetic. In this study, no difference was present in mean glycosylated serum protein level when 11 normal and eight diabetic pregnant women were compared ($p = 0.79$).

The correlation between degree of abnormality in 1-hour 50 gm glucose screening tests and serum glycosylated protein was examined in 17 women with abnormal screening results. The poor correlation ($r = 0.185$) suggests a lack of usefulness of this simple procedure as a substitute for screening tests using glucose challenge and blood glucose measurement. These data confirm that glycosylated serum protein measurements, while a useful adjunct to blood glucose determinations in assessment of metabolic control in diabetic pregnant women, are of no use in either screening for or diagnosing gestational diabetes.

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Discussion

DR. GEORGE D. MALKASIAN, Rochester, Minnesota. I think Dr. Bourgeois and his colleagues are to be congratulated for looking for easier ways for all of us to identify problem patients. We need people with this kind of curiosity to look at things that would be of immediate practical application to one's practice. In that regard, I enjoyed reading the paper. It brought to mind a couple of questions regarding the glucose screening tests that are used. Debate remains among people in the field as to what blood level should lead to use of a glucose tolerance test. From this institution a level of 135 mg/dl was reported, some of the literature mentions 140, and in our institution and some of the others, 150 is used. So, just out of curiosity, I wonder whether the results would have been any different had these different screening levels been compared. I have one other question for Dr. Bourgeois. Regarding his level of glycosylated serum protein for the normal patients, as I read through the manuscript, the mean was 10.7, and for those with abnormal glucose screen results, it was 9.4. It was 9.2 for those who had an abnormal glucose tolerance test. It was lower in those who appeared to be problematic or were potentially problematic than in normal women. I wonder whether you have any thoughts or an explanation for that.

DR. BOURGEOIS. Actually, I think both of your questions pertain to the same thing in a sense, namely, that it is difficult to devise a test that is going to be both sufficiently sensitive and specific at the same time. I think our paramount interest is to avoid missing women who are gestational diabetics. That philosophy probably should underlie the way we design our screening test to identify gestational diabetic patients. The level of 135 mg/dl that we use in our institution is purely a result of a paper from Yale suggesting that 150 mg/dl is too insensitive a level. I said that I think that both of these questions pertain to the same thing because we are struggling with the same things when we decide where to establish a cut-off for glycosylated serum albumin testing. We did not find any difference in levels of albumin in comparing diabetic patients to control subjects—9.2 and 9.4 are not statistically different. One could redefine the way one goes about this and manipulate the administration of glucose and the timing of the test and all that sort of thing, and one would find results a little bit different from what we did. However, I still do not think one could screen effectively.

DR. EDWARD E. WALLACH, Baltimore, Maryland. glycosylated proteins, including hemoglobin and albumin, have been used as tools for evaluation of diabetic status. They do so by reflecting blood glucose levels prevailing over a prolonged period of time and are not subject to the rapid fluctuations of blood sugar related to diet or to perturbation with use of a carbohydrate load. Hemoglobin A_{1c} levels remain elevated in

diabetics for many weeks after control of blood sugar levels has been achieved. Glycosylated protein has a much shorter half-life than hemoglobin A_{1c}. The authors raise the question of sensitivity of protein to monitor or screen for increases in blood sugar. Morris et al.¹ from Duke reported that an excellent correlation of glycosylated serum protein with mean fasting blood glucose concentrations during the preceding 7 days and mean postprandial glucose in a group of pregnant diabetic women who monitored glucose levels at home with an Ames Glucometer. The correlation with use of glycosylated hemoglobin and fasting blood glucose and mean postprandial glucose was weaker. The half-life of red blood cells is 120 days, and the half-life of hemoglobin A_{1c} is 17 days. McFarland et al.² from the University of South Carolina concluded that glycosylated serum proteins and glycosylated hemoglobin determinations are not as sensitive as the glucose tolerance test in detecting gestational diabetes mellitus, as presently defined. Shah et al.³ from Pennsylvania concluded that the sensitivity of hemoglobin A_{1c} is too low to serve as a screening test for the entire obstetric population. Bourgeois and his coauthors confirm the conclusions from Duke, Pennsylvania, and South Carolina. Perhaps, however, there are certain gestational diabetics whose abnormal glycosylated protein levels will predict adverse outcome. Do not abandon this technique as a tool for evaluating or monitoring gestational diabetes yet, especially as it pertains to fetal anomalies.

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DR. JAMES M. INGRAM, Tampa, Florida. I think this is work that should have been done and had to be done because a further search for a quick and simple test to detect the gestational diabetic patient must be carried on. This is not necessarily a negative correlation. Dr. O'Brien, whom you heard this morning, pointed out the same thing as did Dr. Wallach: if one would continue to take these determinations and see whether indeed they might correlate later in pregnancy with congenital defects and other complications, this test might still be useful.

III. Proximal stump salpingitis

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Infection arising in the proximal remnants of the fallopian tubes was noted in five patients, occurring at a time quite remote from tubal ligation procedures. Their unusual clinical presentation and treatment are discussed. (*AM J OBSTET GYNECOL* 1986;155:496-500.)

Key words: Salpingitis, tubal ligation, adnexitis, pelvic inflammatory disease

Historically, there have been few articles discussing pelvic infection after tubal sterilization. The majority of infectious complications reported have been temporally related to the operative procedure itself and the immediate postoperative period. This current report documents five cases of "proximal stump salpingitis," presumably unrelated to the operative procedure but more likely related to de novo salpingitis, since they occurred at a time remote from the sterilization procedure itself. The fact that these patients did not present with "classic" symptoms and signs of pelvic inflammatory disease is presumably explained by the remnant of tube being both shortened and blocked by the tubal sterilization.

Case presentations

Patient No. 1, a 38-year-old white woman, gravida 3, para 3, had undergone laparoscopic tubal ligation by cautery 8 months before the present illness. She presented on day 17 of a normal menstrual cycle, with pelvic pain for 4 days. The patient was sexually active. Examination disclosed a 5 cm exquisitely tender right adnexal mass. The white blood cell count was 8600 with normal differential cell count and normal hemoglobin. A pregnancy test was negative. The patient underwent diagnostic laparoscopy and was found to have an intramural abscess of the cornual portion of the fallopian tube and the proximal stump. This abscess was surgically excised and the patient had an uneventful postoperative course. Pathology report revealed acute suppurative salpingitis.

Patient No. 2, a 35-year-old white woman, gravida 3, para 3, had undergone postpartum Pomeroy tubal sterilization approximately 5½ years before the present illness. She presented on day 7 of a normal menstrual cycle, with a 1-week history of right lower quadrant pelvic pain. The right cornual area of the uterus and parametrial area revealed an exquisitely tender, poorly defined 4 cm right adnexal mass. The white blood cell count was 7200/cm with 64% polymorphonuclear leukocytes and 28% lymphocytes. Hemoglobin was normal; diagnostic laparoscopy was performed and revealed omental adhesions to the right tubal stump. On manipulation of this portion of the omentum, purulent material drained from the proximal stump of the fallopian tube. A right salpingectomy was performed and the postoperative course was uneventful. The patient was treated for 7 days with postoperative oral Vibramycin. The pathology report revealed acute salpingitis.

Patient No. 3, a 42-year-old divorced black woman, had undergone postpartum Pomeroy tubal ligation 8 years previously. She had symptoms of recurrent menometrorrhagia despite two previous curettage procedures. The patient's principal symptoms were those of menometrorrhagia and diffuse pelvic pain with increasing dysmenorrhea. Examination disclosed no masses in the pelvis but a 6-week-size boggy, soft, and tender uterus. The patient elected to have a vaginal hysterectomy and the white blood cell count on admission was 7300/cm with a normal differential count. Hemoglobin was

normal. At the time of operation, the patient was noted to have acute salpingitis, and the pathology report disclosed acute salpingitis and adenomyosis (the preoperative diagnosis), but no acute, subacute, or chronic endometritis was noted in the endometrium. The patient's recovery was uneventful. She was treated with oral Vibramycin the night before operation, 100 mg of Vibramycin intravenously in the recovery room, and 100 mg of Vibramycin intravenously 12 hours later. Long-term follow-up has been uneventful.

Patient No. 4, a 23-year-old married, white woman, gravida 3, para 3, had undergone laparoscopic tubal cauterization 2 years before the present illness. The patient was having hypermenorrhea and metrorrhagia and underwent a curettage. The pathology report showed mild adenomatous atypical hyperplasia and chronic endometritis. The patient was treated with oral Provera and oral Vibramycin, but 5 months later she still complained of menometrorrhagia. She was also noted to have increasing tenderness in the right adnexa in the region of the right cornu of the uterus. Because of the recurrent symptoms and the patient's previous sterilization, she elected to undergo abdominal hysterectomy. At the time of operation, white blood cell count on admission was normal but with 86% polymorphonuclear leukocytes. Preoperative pelvic examination also showed a 5 cm right adnexal mass. At the time of operation, she was found to have a 6 cm follicular cyst in the right ovary, and she underwent a right salpingo-oophorectomy. The pathology report revealed acute salpingitis and follicular cyst of the right ovary. The postoperative course was uneventful. The patient was treated preoperatively with doxycycline and was electively treated for 10 postoperative days with 100 mg of doxycycline orally each day. Long-term follow-up was unremarkable.

Patient No. 5, a 30-year-old married black woman, gravida 2, para 2, had undergone laparoscopic tubal cauterization 29 months earlier. She was admitted to the hospital by her internist, with a chief complaint of lower abdominal pain. On day 6 of the present illness, gynecologic consultation was obtained. Pelvic examination at that time showed bilateral tenderness in the proximal area of both fallopian tubes. The white blood cell count was 12,000/cm with 74% polymorphonuclear leukocytes, 2% band cells, and 20% lymphocytes. It was elected to treat the patient with intravenous Vibramycin with a presumptive diagnosis of proximal stump salpingitis, and after 2 days of intravenous Vibramycin, the white blood cell count was 8800/cm and she was feeling markedly improved. Oral Vibramycin was continued for a total of 14 days with complete resolution of all symptoms. Follow-up since that time has revealed no sequelae and no recurrent pelvic pain.

Comment

A computer-assisted literature review was performed to seek other cases of similar circumstances. The MESH words used were tubal ligation, tubal sterilization, adnexitis, salpingitis, and pelvic inflammatory disease. Several authors have reported on infection as a consequence of various tubal sterilization methods. Loffer and Pent¹ did a review for *Obstetrical and Gynecological Survey* in July of 1975, and they reported that infection occurred in 1.4 per 1000 cases. Most of these were wound infections or stitch abscesses; however, the cases were not broken down as to whether they were tubal sterilizations or diagnostic laparoscopy procedures.

Cheng and Rochat² reported on the safety of combined abortion and sterilization, in which they noted

that pelvic inflammatory disease occurred in 0.3% of combined procedures compared with 1.6% of abortion-only procedures. Their method of diagnosing pelvic inflammatory disease was not discussed. In an article entitled "Does Sterilization Prevent Pelvic Infection?" Hajj³ reported the experience at Chicago Lying-In Hospital. He states: "Over 3500 laparoscopic sterilizations have been performed in a population the majority of which is indigent and relatively stable. To date, none of those patients have been readmitted with the diagnosis of acute pelvic inflammatory disease."

In 1946, Falk⁴ suggested that the division of the fallopian tubes would interrupt the surface migration of bacteria and thereby prevent reinfection. He applied the technique of proximal transection in more than 1000 patients, and he recorded no recurrence of salpingitis in any of his patients so treated.

Sporadic cases of pelvic inflammatory disease in patients with sterilizations have been reported. Poma⁵ reported from Rush Medical College that during a 7-year period only one patient was admitted with pelvic inflammatory disease after bilateral tubal ligation. She was a 23-year-old, para 6 woman who had undergone a postpartum Pomeroy procedure 7 months before. She rapidly improved with medical therapy. LoBue⁶ reported a case of severe pelvic inflammatory disease and peritonitis 36 to 48 hours after Falope ring tubal ligation. Her review included eight other reported cases of pelvic inflammatory disease or abscess formation, all of which occurred between 24 hours and 4 weeks from the Falope ring application. Layde et al.⁷ reported on 282 women undergoing interval tubal sterilization by laparotomy, three of whom were rehospitalized for pelvic abscesses. The time interval between procedure and complication, not stated in the article, was implied to be closely related to the surgical procedure.

The only report that bears similarity to the current report is one by Semchyshyn,⁸ who reported from Toronto in the *Canadian Medical Association Journal*. He described a 30-year-old, para 3 black woman who had fallopian tube stump perforation secondary to abscess formation, 18 months after laparotomy and tubal ligation. His case report, described in a letter to the journal, appears to resemble the cases discussed above.

The cases presented here differ in many respects from those in previous reports. The majority of these patients complained of pain and were found to have exquisite adnexal tenderness out of proportion to the other clinical parameters. The failure to manifest an elevated white blood cell count is very interesting, in view of the pathology reports of acute salpingitis with suppurative material present. Because of the exquisite tenderness in the absence of other clinical signs of infection (fever or white blood cell count elevation), laparoscopic evaluation was chosen to facilitate diagnosis. Surgical management was chosen in three cases because the natural history of proximal stump salpingitis has not been previously described. The patient who was treated with intravenous antibiotics was the most recent one, and the decision to use antibiotics was based on

past experience with this type of illness. The third patient was noted to have salpingitis at the time of hysterectomy and the operative findings were not expected to show salpingitis.

Classic teaching in gynecology has emphasized that pelvic inflammatory disease is extremely unlikely in patients who have had tubal sterilization procedures. The hypothesis is that pelvic inflammatory disease is prevented by the occlusion of the fallopian tube in the proximal portion. What has not been emphasized in the past is that the remnant of proximal fallopian tube is still susceptible to salpingitis, which is why it seemed plausible to call this entity "proximal stump salpingitis." This implies that the infection can spread no further than the occluded tubal stump, unless spontaneous rupture of the stump occurs because of abscess formation.

It is interesting that only one of the patients had a change in the differential cell count as a possible clue to infection. There are several limitations that are noted in reviewing the management of these patients. There are no preoperative culture data, because culdocentesis was not used in the preoperative assessment of these patients. The four patients who had surgical management had cultures done on the specimens, and all reported no growth. Presumably this is because of sterile abscess formation or more likely because of anaerobic infection. The failure to grow anaerobes in clinical specimens has been a problem not only in our laboratory but also for investigators elsewhere.

All patients were afebrile on admission. Again, this absence of clinical evidence of infection led to the prompt surgical evaluation of these patients with laparoscopy. Erythrocyte sedimentation rates were not done but presumably could have been elevated. It would be helpful in the future for patients to have erythrocyte sedimentation rate evaluation to assist in the assessment of this condition.

It is noted that these patients did not require extremely sophisticated antibiotic therapy postoperatively to facilitate recovery. The benign postoperative course of these patients and the rapidity of healing led to the decision to use medical therapy in the fifth patient. It is unclear whether intravenous and oral antibiotic therapy will be uniformly successful, since there is some concern about the alteration in blood supply to the proximal stump after tubal cauterization procedures. If this is a relatively devascularized area, antibiotic therapy may not be successful in all patients. This study serves to remind us that the proximal remnants of the fallopian tube are still vulnerable to ascending infection and that proximal stump salpingitis can disguise itself as an aborted case of pelvic inflammatory disease. The very fact that the fallopian tube is occluded makes the diagnosis of proximal salpingitis more difficult. Since purulent material is unable to obtain access to the pelvic peritoneum, diffuse parametritis is less likely to occur and culdocentesis is extremely unlikely to retrieve purulent material for bacteriologic study. Future man-

agement of such patients may be assisted by erythrocyte sedimentation rate measurement, endometrial biopsy for observation of endometritis or lymphocytic aggregates, endometrial washings for culture, and early laparoscopy for differential diagnosis. Surgical excision may not be required as it may be possible to sterilize the proximal stump with systemic antibiotics. Hopefully, description of this entity will lead clinicians to be suspicious for this disease in patients with tubal sterilization and unexplained pelvic pain.

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Discussion

DR. PAUL B. UNDERWOOD, Charlottesville, Virginia. Thank you very much for bringing to our attention something that really does occur. It is true that people have not written about this as an individual report. I have seen several cases of this type of infection over the years. If you think of the pathogenesis of tubal infection, especially the ones related to gonococcus or to *Chlamydia*, the mode of infection is through the uterus to the fallopian tube. Therefore, if the fallopian tube has been interrupted by tubal ligation, then the proximal part of the tube should become infected or the endosalpinx should be involved, and the distal portion of the tube, which has been separated from the proximal portion, should not. The cases that I have seen have been similar to those you have reported; that is, they do not have the acute infection type of process that you think of with salpingitis with a high fever and high white blood cell count. The only thing you did not mention that has been characteristic to me is that when you examine these women there are tender nodules right on each cornual area of the uterus. Sometimes I have jokingly referred to them as "bull horns." These tender nodules at each cornual area should tip you off to the diagnosis. I think there are several things in salpingitis that have changed in the past year or two. One is that when we start to treat this disease we must remember that we are not treating just gonorrhea, as we thought in the past. At least half of these women are going to have *Chlamydic* infections also, so an antibiotic should cover not only gonorrhea but also *Chlamydia* and, if you are treating a patient who has been symptomatic more than 48 hours, it should also

cover anaerobic organisms. So I think our antibiotic coverage has certainly changed in the past couple of years. The other thing that should be brought out is that laparoscopy definitely plays a big role in salpingitis. If you have a patient that has typical salpingitis, you are going to get some disagreement, but I see no reason to perform laparoscopy in these patients. If they respond as they should, then you have saved them an operation. However, we have certainly, in the past 9 months, found that when a patient does not have typical salpingitis or there is something that does not fit into the picture, a high percentage of patients do not have salpingitis. One of ours who underwent laparoscopy had a cholecystitis; another one had an ectopic pregnancy with a negative pregnancy test, and one had appendicitis. So I would emphasize that in a typical case of salpingitis, the liberal use of the laparoscope, to be sure you are not dealing with some other intraperitoneal infection, is indicated. I would like to make one other comment about salpingitis. The old adage that acute salpingitis with a tuboovarian abscess has to be treated with pelvic "cleanout," that is, removal of both ovaries, tubes, and uterus, and that is the only way you can treat it and get it well, is not 1986 medicine. With modern-day antibiotics you can certainly treat salpingitis, even severe disease, in a conservative manner and some of these women will become pregnant, even though the pregnancy rate is low. Even a tuboovarian abscess, if it is localized, can be excised with preservation of the other ovary and tube.

DR. GEORGE D. MALKASIAN, Rochester, Minnesota. I think there is one other thing that might be useful in evaluating these patients and I am back to ultrasound again. It was noted at the time of laparoscopy that there were cystic masses in each of these, but in only either two or three of the instances, if I remember correctly, were they palpable clinically. Ultrasound evaluation might be of some use in identifying these ahead of time. In light of the fact that all of these patients had had tubal occlusion procedures before this, only two of them underwent hysterectomy. I do not think removal of the ovaries is necessary, but I wondered why the choice to maintain the uterus in place as opposed to removing it at the time of definitive operation.

DR. EDWARD E. WALLACH, Baltimore, Maryland. Thank you for putting these cases together and giving me the opportunity to review them. Five cases of proximal stump salpingitis with the criteria being first the occurrence of the problem at a time remote from the time of the operative procedure and second the absence of classic symptoms and signs of pelvic inflammatory disease. In fact, of the five patients none was febrile. Only one had leukocytosis and in that case the diagnosis was not established pathologically as in the other four. The lapsed time since the sterilization ranged from 8 months to 8 years. Three patients were sterilized by cautery. Two patients had postpartum Pomeroy sterilizations, and only three of the patients had a palpable mass. One of these proved to have a follicle cyst to account for the mass. I think it points up several questions that I have raised in the past. I have always wondered about the patient that we see in the emergency room or hear about having been seen in the emergency room, who has been treated for pelvic inflammatory disease and released on a regimen of antibiotics with a diagnosis of pelvic inflammatory disease but has been afebrile. In the back of my mind I worry about the things that Dr. Underwood mentioned and that maybe we are not dealing with pelvic inflammatory disease. In at least four of these five cases you have documented the presence of salpingitis. The second issue is that salpingitis in the proximal stump occurs the same way as salpingitis would

occur with the tube intact, through surface epithelial extension, but there is less tube to be involved. I also wonder whether the fact that there is usually no mode of egress to the peritoneal cavity lessens the blow of the insult. With the tube intact or a chronically inflamed tube, there may be abscess formation earlier or there may be a pelvic peritonitis. The third aspect that I think is of interest is that Dr. Donald Woodruff has examined a number of proximal portions of the fallopian tube during the past 5 years to look for endometriosis. He has found a significant amount of endometriosis in the proximal stump, which he attributes to blockage of retrograde flow of the endometrium. So there are abnormalities of the distal portion of the proximal stump, which may lend itself to abscess formation as you described here. I would just like to summarize some thoughts by emphasizing that female sterilization is the most widely used contraceptive method in the world today. Most agree that there is no evidence that voluntary female sterilization causes long-term problems. In fact, what you have seen here is not a long-term problem. It may be unrelated to the sterilization, but there is one issue that we have to address. Major complications occur in <2% of all procedures. Delayed complications that require readmission to the hospital occur after <1% of sterilization procedures. Several studies have suggested that women, after sterilization, are prone to a variety of gynecologic problems. In one of the largest prospective studies to date, the Oxford Family Planning Association study, no significant differences were noted after 6 years between 2200 sterilized and 3500 unsterilized women in rates of hospital referral for prolapse of the uterus, menstrual disorders, pelvic inflammatory disease, hysterectomy, or curettage. Rates of hysterectomy may be higher in the previously sterilized person because of a greater willingness of the patient to undergo a hysterectomy or the greater willingness of the surgeon to offer the procedure. In these five patients, I wonder, had they not had sterilization before, whether the two who did undergo hysterectomy would have been treated for pelvic inflammatory disease if the tubes had been intact. I think we all fall into that trap of extirpation a little bit more freely in the previously sterilized patient than in the nonsterilized patient.

DR. JAMES M. INGRAM, Tampa, Florida. I am sure that if Dr. Faulk could be here he would put a halo around your head for writing this article. Many of us have listened to him for about a decade advocate a proximal salpingectomy or resection of the tube as a preventative of salpingitis before the advent of antibiotics. Is it correct that all cultures were negative and all patients responded to doxycycline?

DR. FLETCHER. In the cases that were treated with salpingectomy one or two postoperative doses of doxycycline were used prophylactically, which I will talk about in regard to definitive therapy. The one patient who elected to have medical therapy was treated with Vibramycin with the idea that it would be a chlamydial or mixed infection. That one patient happened to have active rheumatoid arthritis at the time and had been hospitalized recently for that. She was obese and on clinical grounds was selected to be treated; she did so well we let it go at that. She probably had a white blood cell count elevation because of the infection and not the arthritis, since 48 hours later she was without any leukocytosis. With regard to Dr. Underwood's comment about the nodules at the cornu, I am not sure I could palpate nodules except that the patient who received intravenous antibiotics had unilateral findings. Although I did not feel any true nodules on the cornua, I was impressed that in retrospect none of these patients had a palpable mass that so many patients with pelvic inflammatory

disease and ectopic pregnancy seem to have. Why that would be I am not sure; I guess it has something to do with the alteration in the mesosalpinx from the sterilization itself. Liberal laparoscopy was used, as you mentioned, because the results were atypical. We have had extremely poor results in our hospital laboratory in growing anaerobes and in *Chlamydia* testing. It takes approximately 48 to 72 hours to get results, so that these tests were impossible in the first few patients we had. As for the question about sonar, I think it is true that it would be helpful, but we have not found that use of ultrasound has been particularly helpful in localizing fluid in patients with early pelvic inflammatory disease. However, I cannot argue that it might be very helpful in avoiding laparoscopy and in choosing antibiotics as an initial therapy. A hysterectomy was performed in one patient because she had gynecologic symptoms with recurrent menometrorrhagia in spite of having had a previous curettage. The other patient who had hysterectomy had had atypical adenomatous hyperplasia and endometritis at initial curettage. She elected to have conservative therapy with Provera and Vibramycin and within 6 months had recurrence of her menometrorrhagia despite progestational

therapy. At that point she elected to have and the salpingitis was an incidental ser. The one patient I mentioned with the white elevation had also other systemic disease.

DR. MALKASIAN. In your review of the of these cases, namely, the initial sterilization p run across any evidence that there might hav pelvic abnormality at the time of sterilizati sions or other evidence of preexisting pe disease. In those patients in whom a piece of namely, those with the Pomeroy procedur chance to review the piece of tube pathol there was any evidence of tubal abnormali tube?

DR. FLETCHER. In the patients who had lopian tubes, operative reports were availab no comments about peritubular adhesions, postoperative appendectomy adhesions. In who had Pomeroy procedures, I did not loc features in the segments to see if there had b lymphocytes or signs of chronic infection.

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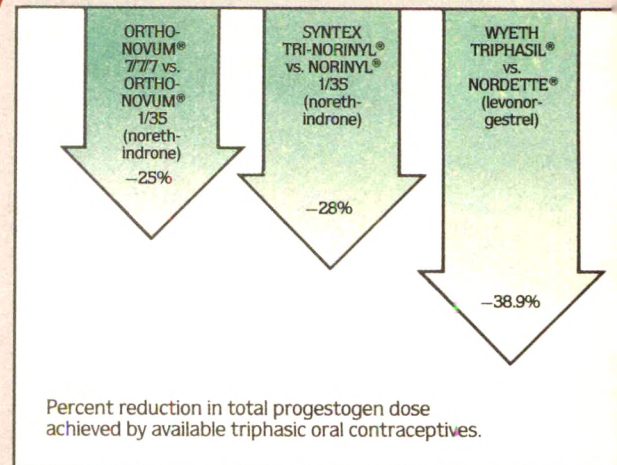
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References: 1. Data on file, Wyeth Laboratories. 2. Gaspard UJ, et al: *Contraception* 31:395-408, April 1985. 3. Vermeulen A, Thiery M: *Contraception* 26:505-513, 1982. 4. Harvenge C, Desager JP, Lecart C: *Curr Ther Res* 33:385-393, Mar 1985. 5. Skouby SO, et al: *Am J Obstet Gynecol* 153:495-500, Nov 1985. 6. März W, et al: *Obstet Gynecol* 153:287-293, Oct 1985. 7. Briggs MH in Brosens I, ed: *New Considerations in Oral Contraception*. Proceedings of an international symposium, Leuven, Belgium (1981), pp 131-151, 1982. 8. Cullberg G, et al: *Contraception* 26:229-243, Sept 1982. 9. Schijf CPT, et al in Harrison RF, et al, ed: *Advances in Fertility Control and the Treatment of Sterility*, MTP Press Ltd, Lancaster, England, pp 61-69, 1984. 10. Larsson-Cohn M, et al: *Fertil Steril* 35:172-179, Feb 1981. 11. Survey results on file, Wyeth Laboratories.

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VERMEULEN ³	6	↔	↔	↔	↔
HARVENGT ⁴	6	↔	↔	↔	↔
SKOUBY ⁵	6	↔	↑	↔	↔
MÄRZ ⁶	3	↔	↑	↔	↔
BRIGGS ⁷	18	↔	↑	↔	↔
CULLBERG ⁸	6	↔	↔	↔	↔
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Indications and Usage—TRIPHASIL is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

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Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suggested association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke. OC users who are 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclampsia toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 3 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death during intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of oral estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence

from well-controlled studies that progestogens are effective. There is some evidence that polydomy and possibly ot types of polydomy are increased among abortions from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of the missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects**—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. **Elevated Blood Pressure**—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in use increases, however, with longer exposure, and in the 5th year of use is 2 1/2 to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. **Headache**—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—**GENERAL**—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depressive recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

- Increased sulfobromophthalen retention.
- Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
- Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.
- Decreased pregnandiol excretion.
- Reduced response to metyrapone test.

Information for the Patient—See Patient Package Leaflet.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

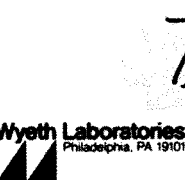
The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice, migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, catarracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. If (Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.



Maternal-perinatal outcome associated with the syndrome of hemolysis, elevated liver enzymes, and low platelets in severe preeclampsia-eclampsia

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During an 8-year period, 112 severe preeclamptic-eclamptic patients with the above syndrome were studied. The incidence of this syndrome was significantly higher in white patients, in patients with delayed diagnosis of preeclampsia and/or delayed delivery, and in multiparous patients. Twenty-six patients had amniocentesis and 16 received epidural anesthetics. There was one maternal bleeding episode associated with epidural anesthetics. The use of steroids in 17 patients did not improve maternal platelet count. The overall perinatal mortality was 367 per 1000 and neonatal morbidity was significant. There were two maternal deaths and two patients with ruptured liver hematoma, and nine had acute renal failure. Thirty-eight percent had intravascular coagulopathy and 20% had abruptio placentae. On follow-up, 44 patients used oral contraceptives without maternal morbidity and 38 patients had 49 subsequent pregnancies. Only one patient had recurrence of the syndrome in subsequent pregnancies. The presence of a "true" syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome) in preeclampsia is associated with poor maternal-perinatal outcome. (*AM J OSTET GYNECOL* 1986;155:501-9.)

Key words: Severe preeclampsia, HELLP syndrome, perinatal outcome

There is considerable controversy regarding the nature, incidence, clinical significance, and management of the syndrome of hemolysis, elevated liver enzymes, and low platelets in severe preeclampsia-eclampsia. Goodlin et al.¹ described this syndrome as EPH (edema, proteinuria, hypertension) gestosis type B and claimed that this syndrome had been reported in the obstetric literature for about 100 years. Weinstein^{2,3} considered this syndrome as a unique variant of severe preeclampsia and called it the HELLP syndrome in reference to the laboratory abnormalities. Almost all previous reports¹⁻⁵ described the syndrome in white patients, while the occurrence among black patients has been very low.⁶ Management of these cases has been conflicting, with some authors^{2,3,7,8} recommending immediate delivery and others^{1,4,5} recommending a more conservative active approach to prolong pregnancy in an attempt to achieve better fetal maturity.

We report the observations from 112 recently managed cases of severe preeclampsia-eclampsia compli-

cated by the "HELLP syndrome." The purpose of this clinical report is (1) to determine the influence of several maternal obstetric factors on the frequency of this syndrome, (2) to assess the relationship between this syndrome and maternal perinatal outcome, (3) to report the previous obstetric and subsequent pregnancy outcome, and (4) to report the potential risks of using amniocentesis, epidural anesthetics, and oral contraceptives in such patients.

Material and methods

The E. H. Crump Women's Hospital and Perinatal Center, Memphis, Tennessee, serves as the tertiary referral center for approximately 30,000 deliveries annually in the Mid-South. The area includes five states: Tennessee, Arkansas, Mississippi, Missouri, and Kentucky. During the 8-year period from August 1, 1977, to July 31, 1985, 1153 of the pregnancies delivered at this center were complicated by severe preeclampsia (n = 967) or eclampsia (n = 186). The presence of hemolysis, elevated liver enzymes, and low platelets (HELLP) was demonstrated in 112 patients for an incidence of 9.7% in severe preeclamptic-eclamptic pregnancies.

The chart of each patient was studied to delineate any potential prenatal events leading to the development of this syndrome. Every effort was made to obtain all prenatal, emergency room, and hospital records of patients referred from local hospitals and clinics. The

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Table I. Clinical characteristics

	No.	%
Maternal age (yr)		
≤25	64	57
26-30	30	27
>30	18	16
Race		
Black	64	57
White	48	43
Gravidity		
1	58	52
≥2	54	48

analysis focused on the influence of delayed diagnosis of preeclampsia and delayed delivery, maternal race, parity, and associated medical and obstetric complications on the frequency of this syndrome. Additional data included previous pregnancy outcome, subsequent pregnancy outcome, and safety of using oral contraceptives in such patients. The influence of amniocentesis, use of steroids, type of anesthesia, mode of delivery, and fetal gestational age on both maternal and perinatal outcome was also analyzed.

All patients had initial blood samples obtained within 2 hours of admission and these tests were repeated as indicated. Laboratory evaluation included coagulation profile, liver function tests, and renal function tests.⁹ In addition, fluorescent antinuclear antibodies and bleeding times were determined in some of these patients. All patients had a platelet count $<100 \times 10^3/\text{mm}^3$, hemolysis as determined by peripheral smear, increased bilirubin and lactic dehydrogenase, and increased serum glutamic oxaloacetic transaminase. All of these levels were more than 3 SD from the normal mean at this hospital.⁹ Disseminated intravascular coagulation was defined as the presence of thrombocytopenia, low fibrinogen levels (plasma fibrinogen $<300 \text{ mg/dl}$), and fibrin split products $>40 \mu\text{g/ml}$. Only values obtained before delivery were considered in the diagnosis of the syndrome.

Additional tests used included ultrasonography, computerized tomographic scan of the abdomen ($n = 4$), bone marrow biopsy ($n = 5$), and amniocentesis for fetal lung maturity ($n = 26$). Intravenous magnesium sulfate with or without hydralazine was used to stabilize patients for a minimum of 4 hours before induction of labor or abdominal delivery in all but 12 patients who received corticosteroids to enhance fetal lung maturity. In these latter patients, delivery was delayed for 48 hours. All patients had electronic fetal heart rate monitoring during labor. Epidural anesthesia was used in 16 patients. The other patients received local infiltration anesthetics for vaginal delivery and balanced general anesthetics for cesarean section. The level of plate-

let count in the mother did not influence the mode of delivery of the infant.

Infant clinical data included gestational age, birth weight, Apgar score, and neonatal complications. In addition, neonatal hematologic data were obtained in 80 infants either from cord blood or after admission to the neonatal intensive care unit. Neonatal thrombocytopenia was defined as a platelet count $<150 \times 10^3/\text{mm}^3$, and leukopenia was defined as a white blood cell count $<5000/\text{mm}^3$.

Results

Table I summarizes the clinical characteristics in these patients. The mean maternal age was 24.8 ± 3.1 years (range 14 to 40 years). This group had a significantly higher maternal age ($p < 0.001$) than the other patients who did not have this syndrome (mean age 18.9 ± 4.7 years). In considering all patients with severe preeclampsia-eclampsia, the incidence of this syndrome was significantly higher in white (24.5% versus 6.9%, $p < 0.0001$) and multigravid (14% vs 7.6%, $p < 0.0001$) patients. Sixteen patients (14.3%) had chronic hypertension, two patients (1.8%) had diabetes, and one patient (0.9%) had lupus nephritis. None of these were significantly different when compared with the group without the syndrome.

Table II summarizes the previous obstetric outcome in the 54 patients with previous pregnancies. These patients had a total of 107 pregnancies for an average of two pregnancies per patient (range 1 to 7). Excluding the abortions, the perinatal mortality in previous pregnancies was 16.3%.

At the time of admission to a hospital, 101 (90%) had epigastric and/or right upper quadrant pain, 50 (45%) had nausea and/or vomiting, and 56 (50%) had headache. Sixty-two (55%) had demonstrable edema, 95 (85%) had significant proteinuria ($\geq 2+$) on dipstick, 10 (9%) had minimal proteinuria (1+), and 7 (6%) had no proteinuria. Fourteen patients (14.5%) had a diastolic blood pressure of $<90 \text{ mm Hg}$ (range 74 to 88 mm Hg), while 77 (68.8%) had a diastolic pressure $\geq 110 \text{ mm Hg}$.

Laboratory findings. Table III summarizes the laboratory findings before delivery in all patients. In addition to these findings, all patients had lactic dehydrogenase above 600 IU/L and all of them had an abnormal peripheral smear. Forty-two patients (38%) had disseminated intravascular coagulation (low platelets, low fibrinogen, and positive fibrin split products), and 24 (21.4%) had prolonged prothrombin time and partial thromboplastin time. Most of these complications were associated with abruptio placentae and/or fetal death. Bleeding time was determined in 21 patients and was abnormal (>15 minutes) in 14 (67%). Fluorescent

Table II. Previous obstetric outcome in multigravid patients (n = 54)

Outcome	No.	%
Preeclampsia/eclampsia	26	24.3
Abruptio placentae	5	4.8
Stillbirths	15	14.0
Neonatal deaths	3	2.8
Spontaneous abortion	15	14.0

antinuclear antibodies were tested in 62 patients and results were positive in two only. One of these patients had lupus nephritis.

The average creatinine clearance was 55 ± 33 ml/min (range 0 to 138). Nine patients had acute renal failure with an average creatinine clearance of 7.6 ± 5.8 ml/min (range 0 to 20). The pregnancies of these nine patients were complicated by one or more of the following: abruptio placentae, fetal death, or disseminated intravascular coagulation.

Management and maternal outcome. Sixty-five patients (58%) were maternal referrals from local hospitals and clinics, 26 (23%) had prenatal care at our clinics, and 21 (19%) had no prenatal care. Delayed diagnosis of preeclampsia and/or delayed delivery were evident in 52 (57%) of the patients who had prenatal care. The average delay was 8 days (range 3 to 22 days). Most patients with delayed diagnosis had misdiagnoses of conditions other than preeclampsia. These conditions included gastroenteritis, hepatitis, gallbladder disease, pyelonephritis, chronic renal disease, and idiopathic thrombocytopenic purpura. Five patients had bone marrow aspirates that revealed increased megakaryocytes for which they were treated with prednisone.

Ultrasound examinations of the liver were performed in 82 of these patients with the diagnosis of subcapsular hematoma of the liver suspected in four. This diagnosis was later confirmed in two of the four patients. One was confirmed by liver-spleen radionuclide scan, while the second was confirmed by abdominal computerized tomographic scan. Both procedures were performed during the postpartum period following vaginal delivery. The remaining two patients had negative abdominal computerized tomographic scans.

Twenty-six patients had amniocentesis for fetal lung maturity. The mean platelet count at the time of amniocentesis was $59 \pm 26 \times 10^3/\text{mm}^3$ (range 12 to $98 \times 10^3/\text{mm}^3$). There was no maternal bleeding associated with this procedure. Twelve of the 26 patients received either dexamethasone or betamethasone to stimulate fetal lung maturity. Overall, 17 patients received steroids for either suspected idiopathic thrombocytopenic purpura or fetal lung immaturity. Three of these 17

Table III. Laboratory findings

Test	Mean $\pm$ 1 SD	Range
Platelet count $\times 10^3/\text{mm}^3$	55 ± 23	9 – 98
Initial hematocrit (%)	35.8 ± 5.5	12 – 48
Postpartum hematocrit (%)	25.3 ± 3.9	15 – 35
Creatinine (mg/dl)	1.7 ± 2.0	0.7 – 10.9
Uric acid (mg/dl)	8.3 ± 3.1	4.8 – 15.2
Serum glutamic oxaloacetic transaminase (U/L)	434 ± 556	72 – 4000
Bilirubin (mg/dl)	2.7 ± 2.5	1.2 – 16.7
Albumin (gm/dl)	2.8 ± 0.4	1.9 – 3.6

Table IV. Maternal complications

Complication	No.	%
Abruptio placentae	22	20
Intravascular coagulopathy	42	38
Acute renal failure	9	8
Pleural effusions	8	7.1
Pulmonary edema	5	4.5
Ruptured liver hematoma	2	1.8
Maternal deaths	2	1.8

demonstrated improvement in platelet count, while the platelet count remained the same or became lower in the remaining patients.

Sixteen patients had epidural anesthesia. The mean platelet count in this group of patients was $83 \pm 8 \times 10^3/\text{mm}^3$. There was one case of bleeding in the epidural space in this group of patients. The catheter was kept in place for 24 hours following delivery, after which the bleeding stopped spontaneously. The platelet count of this patient was $93 \times 10^3/\text{mm}^3$; however, the bleeding time was >15 minutes.

Forty-one patients (36.6%) had vaginal delivery and 71 (63.4%) had cesarean delivery. Indications for cesarean section included fetal distress, malpresentations, previous cesarean section, failed oxytocin induction of labor, and an unfavorable cervix. Three patients required cesarean hysterectomy and 17 had bilateral tubal ligation. Eleven patients (10%) received platelet transfusions before operation because of severe thrombocytopenia (platelet count $< 20 \times 10^3/\text{mm}^3$). Overall 93% of all patients received blood and/or blood products to correct hypovolemia or coagulopathy.

Table IV summarizes the maternal complications in these patients. Disseminated intravascular coagulopathy was the most frequent complication followed by abruptio placentae. Both of these complications were strongly associated with the presence of antepartum fetal death. Eight of nine patients with renal failure had acute tubular necrosis and the other patient presented with acute cortical necrosis in association with

Table V. Perinatal outcome (n = 114 births)

Outcome	No.	%
Perinatal deaths	38	33.3
Stillbirths	22	19.3
Neonatal deaths	15	17.4
Gestational age (wk)		
≤30	47	41.2
31-36	46	40.4
>36	21	18.4
Small for gestational age	36	31.6
Apgar score		
1 min ≤4	39	42.4
5 min <7	26	28.3

abruptio placentae and fetal death at 29 weeks' gestation. Five of the nine patients required hemodialysis or peritoneal dialysis. The patient with cortical necrosis received a kidney transplant.

The two cases of ruptured subcapsular hematoma of the liver occurred in patients who had vaginal delivery of stillborn infants at 39 and 23 weeks' gestation. Both patients had delayed diagnosis and/or delivery for 3 days only. Both patients had surgical evacuation and drainage of the hematomas in the postpartum period. The hospital course was complicated by pleural effusions, disseminated intravascular coagulation, and pulmonary edema. However, both of them had normal hematologic and liver function tests at the time of discharge from the hospital. Both had subsequent pregnancies without complications.

One maternal death occurred in a patient with antepartum eclampsia at 39 weeks' gestation. The patient was referred in a private car and had several convulsions during transport resulting in cardiac arrest at the time of arrival to the perinatal center. She was resuscitated and had cesarean section for fetal distress with delivery of a live term infant with good Apgar scores. The hospital course was complicated by deep coma, disseminated intravascular coagulation, pulmonary edema, and pleural effusions. She had a flat electroencephalogram immediately post partum that persisted until death 9 weeks later. Autopsy permit was denied. The second death occurred in a severely preeclamptic patient who had cesarean section for a premature breech at 26 weeks' gestation. During operation the patient had a cardiac arrest from severe hypoxia secondary to esophageal intubation. She had decerebrate rigidity and a flat electroencephalogram immediately post partum. She died 5 days later and autopsy was denied by the family.

Forty-four patients received oral contraceptives for a minimum of 2 months. None of them had evidence of HELLP syndrome while using the pills. Thirty-eight patients had 49 subsequent pregnancies. One patient (2.6%) had the HELLP syndrome in two subsequent

pregnancies. However, both pregnancies were complicated by abruptio placentae and fetal death. The remaining patients did not have recurrence of this syndrome in any of their subsequent pregnancies.

Perinatal outcome. The 112 pregnancies resulted in 114 births (two sets of twins). There were 22 stillbirths and 16 neonatal deaths for a perinatal mortality rate of 367 per 1000. Table V summarizes the perinatal outcome in these patients. The mean gestational age was 31.8 ± 2.5 weeks (range 20 to 41 weeks), and the mean birth weight was 1524 ± 780 gm (range 300 to 3487 gm). However, in the neonatal deaths, the mean gestational age was 26.7 ± 1.9 weeks (range 23 to 30 weeks), while the mean birth weight was 698 ± 232 gm (range 310 to 1140 gm). Both of these were significantly lower ($p < 0.0001$) than those in surviving babies. In addition, infants who died had a mean 1-minute Apgar score of 2.4 ± 1.7 (range 1 to 6) and a mean 5-minute Apgar score of 3.1 ± 2.3 (range 1 to 7).

Twenty-one infants (26%) had thrombocytopenia (platelet count $<150 \times 10^3/\text{mm}^3$), 11 (14%) had leukopenia and/or neutropenia, and nine (11%) had disseminated intravascular coagulation. All of these abnormalities occurred in premature babies who had low Apgar scores and/or severe growth retardation. There was no correlation between the degree of maternal thrombocytopenia and the development of neonatal thrombocytopenia. In addition, there was no association between the presence of disseminated intravascular coagulation in the mother and that in the neonate.

Comment

The debate concerning the HELLP syndrome in pre-eclampsia-eclampsia has been the subject of several recent reports.^{1-5, 10} A review of the literature highlights the confusion and controversy concerning the definition, diagnosis, incidence, cause, and management of this syndrome. Weinstein^{2,3} considered it a unique variant of preeclampsia, while Goodlin et al.^{1,11} considered it an early form of severe preeclampsia and labeled it impending gestosis or EPH gestosis. On the other hand, Mackenna et al.⁴ considered it as misdiagnosed preeclampsia, while Greer et al.¹⁰ considered it as mild disseminated intravascular coagulation that was missed because of "technical inadequacy." In addition, there are considerable differences regarding the degree of laboratory abnormalities needed to make the diagnosis. The first report by Weinstein² included 29 such patients; however, only 18 had platelet counts $<100,000/\text{mm}^3$ on admission. Eventually all patients had a count below 100,000 with some of those occurring post partum. Only 16 patients had abnormal bilirubin values (level not stated) and all had abnormal serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase values, but the abnormal values were not

mentioned. Hemolysis was defined as the presence of an abnormal peripheral blood smear (burr cells and/or schistocytes), which occurred in 28 of the 29 patients. Weinstein³ later expanded the number to 57 patients; however, he did not elaborate further on the diagnostic criteria. Forty-eight of these patients had an abnormal peripheral blood smear, 62% had hyperbilirubinemia, and only two patients had disseminated intravascular coagulation.

Goodlin et al.¹ reported on 13 patients with "EPH gestosis" or "impending gestosis." All patients had severe thrombocytopenia (platelet count $<75 \times 10^3/\text{mm}^3$), 10 of 11 had marked elevations of serum glutamic oxaloacetic transaminase (range 138 to 690 IU/L), and none had disseminated intravascular coagulation. In a subsequent report, Goodlin¹¹ summarized the findings in 46 patients with type B "EPH gestosis." Ninety-four percent had a platelet count $<75 \times 10^3/\text{mm}^3$ and 96% had a serum glutamic oxaloacetic transaminase value above 30 IU/L.

Mackenna et al.⁶ studied 27 patients with severe preeclampsia-eclampsia who had "HELLP" syndrome. Liver enzymes were stated to be elevated in all patients (levels not mentioned) and only four patients had elevated bilirubin levels. However, only 23% had platelet counts $<100 \times 10^3/\text{mm}^3$ on admission and 77% had such a value at the time of delivery. One patient had disseminated intravascular coagulation in association with abruptio placentae.

Brazy et al.¹² studied 28 patients who had severe preeclampsia-eclampsia before 36 weeks' gestation. Sixteen patients had a platelet count below $150 \times 10^3/\text{mm}^3$ with eight of them having a platelet count below $50 \times 10^3/\text{mm}^3$. All 16 patients had elevated liver enzymes (serum glutamic oxaloacetic transaminase > 50 IU/L, lactic dehydrogenase > 350 IU/L), while three patients had disseminated intravascular coagulation. Thiagarajah et al.⁵ reported on 13 patients with severe thrombocytopenia in association with preeclampsia. All patients had admission platelet counts below $100 \times 10^3/\text{mm}^3$; however, the count was above $100 \times 10^3/\text{mm}^3$ in six patients at the time of delivery. This improvement in platelet count was attributed to the use of corticosteroids. Significant elevations in liver enzymes (serum glutamic oxaloacetic transaminase and lactic dehydrogenase) were evident in eight of these patients. Serum bilirubin levels were elevated in six of eight patients, and an abnormal peripheral smear was present in one patient studied. Three patients had a fibrinogen level below 300 mg/dl. Renal function was compromised in all patients (creatinine clearance 5.3 to 104 ml/min). Five patients had bone marrow biopsies and two had gingival biopsies.

In contrast, the evidence for hemolysis and abnormal liver enzymes was more pronounced in the cases reported by Killam et al.,⁷ Schwartz and Brenner,⁸ Prit-

chard et al.,¹³ McKay,¹⁴ Beecham et al.,¹⁵ and Lopez-Llera et al.¹⁶ The majority of the patients studied in these reports had disseminated intravascular coagulation in association with the hemolysis.

The incidence of this syndrome among patients with severe preeclampsia-eclampsia ranges from 4% to 12%.⁴⁻⁶ However, the true incidence remains unknown considering the above differences in diagnostic criteria. In addition, the incidence of this syndrome is influenced by age, race, parity, region, and duration of the preeclampsia before delivery. The incidence is highest among older, white,¹⁻⁵ and multiparous¹¹ patients. Moreover, the incidence is higher in preeclamptic patients with delayed diagnosis and/or delayed delivery.¹⁻⁵

The patients studied in this report are a well-defined group with documented evidence of hemolysis, elevated liver enzymes, and low platelets. All patients had the abnormalities before delivery and each patient had true hemolysis proved by abnormal peripheral smear, elevated bilirubin values (mainly indirect), and marked elevations in lactic dehydrogenase. In addition, all patients had significant thrombocytopenia and marked elevations in serum glutamic oxaloacetic transaminase values. Consequently, 38% of them had disseminated intravascular coagulation and 21% had prolonged prothrombin and partial prothrombin times. It is conceivable that this high incidence of coagulopathy has been influenced by the high percentages of abruptio placentae (20%) and fetal death (19%). In fact, there was a strong association between the presence of coagulopathy and abruptio placentae and/or fetal death in such pregnancies. These findings are similar to those reported by some authors^{7, 13-16} but are in disagreement with the findings reported by others.¹⁻⁵ Most of the similarities or differences are related to the diagnostic criteria used to define the "HELLP" syndrome.

The incidence of the syndrome among patients with severe preeclampsia-eclampsia in this study was 9.7%. This high incidence is due to the high percentage of complicated cases of severe preeclampsia-eclampsia referred to the perinatal center. In fact, 58% of patients with this syndrome were maternal referrals and only 21% of the total had prenatal care at our clinic. The incidence was significantly higher in the white patients (24.5%) and in multigravid patients (14%). This finding is in agreement with those reported by others.¹⁻⁵ In addition, the incidence is significantly higher in patients who had delayed diagnosis of preeclampsia and/or delayed delivery. This was evident in 57% of patients who had prenatal care in this study.

In pregnant patients presenting with the signs, symptoms, and laboratory findings of HELLP syndrome, there has been confusion with various diseases and syndromes. This is particularly true in the patient remote

from term whose signs of preeclampsia (that is, hypertension and proteinuria) are unimpressive. This subject has recently been summarized by Goodlin¹¹ and will not be discussed here. Consequently, management of these patients has included various medical regimens and surgical procedures. Some of the modalities used included bone marrow biopsies, liver biopsies, gingival biopsies, kidney biopsies, liver biopsies, and laparotomies. Medical treatment has included plasma volume expansion with crystalloids, albumin, fresh-frozen plasma transfusions, plasmapheresis, dialysis, prednisone, aspirin, antiplatelet substances, heparin, prostacyclin, immunosuppressive drugs, antithrombin III, various antihypertensives, sedatives, and anticonvulsants. The patients in these reports were a heterogeneous group with a wide range of medical and surgical complications as well as a wide range of abnormal laboratory findings at the time of management. Hence it is impossible to draw any conclusions from these reports regarding the potential benefit or risk of the management used.

Some authors^{2,3,7,8} consider the presence of this syndrome as an indication for immediate delivery, while others^{1,4,5} recommend a more conservative approach to prolong pregnancies in cases of fetal immaturity. Weinstein^{2,3} and others^{7,8} supported by the poor pregnancy outcome associated with delaying delivery recommended "expeditious delivery to improve maternal and fetal outcome." On the other hand, Goodlin¹¹ considered hypovolemia as the cause of this syndrome and hence recommended plasma volume expansion with 5% albumin. He reported a 10% success rate in prolonging pregnancies in such patients. In addition, he reported improvement in laboratory findings following the use of prednisone and plasma expansion. Thiagarajah et al.⁵ reported an increase in platelet count and improvement in liver enzyme levels in five patients treated with prednisone or betamethasone. In addition, amniocentesis was performed in five of these patients without bleeding complications. Mackenna et al.⁴ attempted conservative management in 27 patients with this syndrome. Management included bed rest and intravenous magnesium sulfate. Amniocentesis was performed in 21 of the patients without complication. The other six were delivered before the amniocentesis could be done. Seven patients had mature lung profiles and were delivered within 24 hours. Hence only half of the patients actually had conservative management. Liver function tests improved spontaneously in four patients and platelet counts remained stable in some patients. Two patients (7.4%) had abruptio placentae and both resulted in fetal death.

Sixteen patients in this study received epidural anesthetics with one of them developing significant bleeding in the epidural space. We believe that the use of an

epidural anesthetic in such patients is potentially dangerous. Therefore, it should be reserved for patients in whom general anesthesia is more potentially hazardous. Such patients include those with marked laryngeal edema, marked obesity with a short neck, and pulmonary disease.

Twenty-six patients had amniocentesis with an average platelet count of $59 \times 10^3/\text{mm}^3$ at the time of the procedure. None had any bleeding problems. This finding is in agreement with other reports.^{4,5} Seventeen patients received steroids for either idiopathic thrombocytopenic purpura or fetal lung immaturity. Only three patients showed improvement in maternal platelet count. It is possible that this change was a coincidental finding.

Pregnancies complicated by severe preeclampsia-eclampsia and HELLP syndrome are associated with poor maternal and fetal outcome. The reported perinatal mortality ranges from 7.7% to 60%,^{3,5,7} depending on the number of patients and the severity of the disease process. In addition, these patients are at increased risk for maternal mortality and morbidity. Lopez-Llera et al.¹⁶ reported eight maternal deaths (24.2%) among 33 patients with this syndrome while Weinstein³ reported two maternal deaths (3.5%) among 57 patients. Two of the three patients described by Pritchard et al.¹³ died while there was one maternal death among the four patients studied by McKay.¹⁴ Moreover, the mothers are at increased risk for liver rupture, disseminated intravascular coagulation, abruptio placentae, and acute renal failure.

The findings of this study suggest that these pregnancies are associated with poor perinatal outcome. The perinatal mortality rate was 367 per 1000, which is much higher than that reported by others.^{3,5} This difference may be attributable to the fact that all patients in this study had "true" HELLP syndrome while other reports included patients with minimal diagnostic criteria. Most of the perinatal deaths were related to abruptio placentae, intrauterine asphyxia, and prematurity. Forty-one percent of the deliveries occurred at less than 31 weeks' gestation and 32% of the infants were growth retarded. Additionally, 42% of the infants had low 1-minute Apgar scores, while 28% had a low 5-minute score. Twenty-one (26%) of 80 infants had thrombocytopenia and 14% had leukopenia-neutropenia. Most of these abnormalities occurred in sick premature babies. However, there was no correlation between the severity of coagulopathy in the mother and that in the baby. This finding is in disagreement with those reported by others,^{3,12} who suggested a common pathologic immune factor causing these abnormalities.

Moreover, there were two maternal deaths, two patients had ruptured subcapsular hematomas of the liver, and several had significant morbidity (Table IV).

Almost all patients required blood and/or blood product transfusions and five patients required dialysis for renal failure. Consequently, the above findings argue against any potential benefit from delaying delivery in preeclamptic patients with "true" HELLP syndrome. Hence, it is our opinion that most of the controversy surrounding the management and outcome of such pregnancies can be related to differences in populations studied by various authors.

It is of interest to note that 38 of the patients with this syndrome were observed through 49 subsequent pregnancies. Only one patient had recurrence of this syndrome in subsequent pregnancies. This was associated with recurrent abruptio placentae. In addition, 44 patients received oral contraceptives without recurrence of this syndrome. This information should be helpful in counseling and reassuring patients regarding future pregnancies and safety of using oral contraceptives.

In summary, patients with severe preeclampsia and HELLP syndrome constitute a heterogeneous group with a wide range of manifestations and laboratory abnormalities. Management of such patients will depend on several obstetric and maternal variables including fetal gestational age and degree of laboratory abnormalities. The frequency of this syndrome is dependent on several maternal and obstetric factors. Finally the presence of "true" HELLP syndrome in preeclampsia is associated with poor maternal-perinatal outcome.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. L. DANIEL CROOKS, JR., Richmond, Virginia (Official Guest). A thorough retrospective study was conducted in a tertiary referral center in Memphis, Tennessee, by Dr. Sibai and his group. There were 1153 pregnancies complicated by severe preeclampsia or eclampsia delivered at this center during an 8-year period, ending July 31, 1985. One hundred twelve patients, representing 9.7% of those pregnancies reviewed, exhibited the presence of hemolysis, elevated liver enzymes, and low platelets, referred to as "HELLP syndrome" by Weinstein^{1,2} and described earlier by others.³

The incidence of preeclampsia is usually about 5% of all pregnancies.⁴ One would then compute that in an area delivering 30,000 babies annually, 12,000 cases of preeclampsia would be encountered during a similar 8-year period. This would support a frequency in the general population of one per 120 live births. Indeed, the incidence of HELLP syndrome may be much higher because no statistics are available regarding those patients treated elsewhere and never referred to the Memphis center.

The authors report a higher incidence in older, white, multiparous patients with delayed diagnosis and/or delayed delivery, which seems to be in agreement with previous reports by others. This should not be surprising because 58% of the patients reported on were referred from outside the center and were probably patients with prenatal care and private physicians. One can only postulate how many were treated by their physicians in rural or community hospitals and were not referred.

Dr Sibai is to be commended for his strict adherence to criteria for diagnosing HELLP syndrome. Before delivery, all patients to be considered for this study had thrombocytopenia with platelet counts $<100 \times 10^3$

mm³, peripheral blood smears demonstrating hemolysis, and increases in bilirubin, lactic dehydrogenase, and serum glutamic oxaloacetic transaminase levels more than 4 SD from the norm. In his review of the literature, Dr. Sibai describes the considerable differences regarding the degree of laboratory abnormalities. There is small wonder many consider HELLP as an early form of preeclampsia³ or "misdiagnosed preeclampsia."⁵

Obviously, everyone attending the obstetric patient should consider the HELLP syndrome in all preeclamptic patients. It can be easily confused with non-obstetric diagnoses such as gastritis, hepatitis, and gallbladder disease. Greater than 90% had epigastric and/or right upper quadrant pain. Nausea and vomiting are not infrequent. Actually, the laboratory findings may be seen before the signs and symptoms of severe preeclampsia. Perhaps we should routinely order platelet evaluations several times during pregnancy.

While he suggests an aggressive approach and early delivery of his patients, Dr. Sibai nevertheless did 26 amniocenteses and delayed delivery for 48 hours in 12 patients who received corticosteroids to enhance fetal pulmonary maturity. Indeed some authors such as Thiagarajah et al.⁶ believe a more conservative approach to prolong the pregnancy is beneficial to the infant and may improve the maternal thrombocytopenia. Are there any instances in which the authors would delay delivery longer than 48 hours?

Epidural anesthesia was used in 16 patients (14.3%), which is surprising in view of the contraindications of conduction anesthesia with potential coagulation problems such as maternal hemorrhage.⁷ There was one episode of significant bleeding into the epidural space, and Dr. Sibai believes only patients with marked laryngeal edema, obesity with a short neck, and pulmonary diseases should be considered for epidural anesthesia in the future. Arguments could be made for general anesthesia in those selected patients, particularly if they require cesarean sections, in order to protect and provide an adequate airway.

Two maternal deaths were noted in this study and should be mentioned because they apparently were preventable. One patient convulsed in route to the center in a private car, had resultant cardiac arrest, and was brain dead immediately after cesarean section. The second patient suffered severe hypoxia after esophageal intubation, had cardiac arrest, and also had a flat electroencephalogram. If one corrects for these preventable deaths and reviews the literature, there are 231 patients with five deaths.^{2, 3, 5, 6, 8-11} No statistics are available identifying this syndrome as the cause of maternal death in rural or community hospitals, but it could be assumed somewhat higher.

In summary, Dr. Sibai and his group have compiled impressive statistics regarding a well-defined group of patients with the HELLP syndrome. The thorough review of the available literature provides future authors with strict diagnostic criteria and therapy regimens that

hopefully will make conclusions possible and facilitate the comparison of similar studies.

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DISCUSSER NO. 2. I get a little worried when we set criteria for diagnosis. When you see a strict criterion such as a platelet count near 100,000/mm³ and then make the diagnosis, I think a lot of things have gone on before then that you could have made the diagnosis and maybe transported the patient earlier. We should look at the rate of platelet fall. If indeed you have a patient with preeclampsia in labor, then I think serial platelet counts are most important. She may have an initial count of 200,000/mm³, but the next one may be 125,000/mm³. I think the rate of fall is extremely important. If we wait until the diagnosis is made there may be a delay in transport.

DR. SIBAI (Closing). Just 2 days ago, prior to coming here, we had a patient we were giving steroids. It is just amazing that sometimes within minutes you see the platelet counts fluctuate. At one time you are dealing with 125, then suddenly it goes down to 40. This is why in these types of cases we usually keep doing platelet counts, sometimes 2- to 4-hour counts serially. We have to decide if the patient is really sick or do we want to delay delivery. The maximum wait is around 48 to 72 hours just to give steroids, because, as you can see, the pregnancy outcome is really poor. It is evident here, even though we are delaying delivery and improving

gestational stage to get the benefit of steroids, we are delivering babies who are sick. They do not grow in utero, and they are becoming more hypoxic. There are higher incidences of growth retardation and higher incidences of low Apgar scores at 1 and 5 minutes. This is why I disagree that this criterion is an immediate

indication for delivery. I think perhaps you could work with them, if you want to use steroids, and wait 48 to 72 hours, but it is very important that these evaluations be done serially because you see the sudden drop in platelet count just within hours. •

Intestinal injury in gynecologic surgery: A ten-year experience

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From 1973 to 1982, a total of 128 incidents of intestinal injury were recorded in patients undergoing gynecologic operations. Of all lacerations, 37% occurred during entrance into the peritoneal cavity, 35% during lysis of adhesions or performance of pelvic or abdominal dissections, 10% during laparoscopy, 9% during vaginal operations, and 9% during dilatation and curettage and dilatation and evacuation. Injury involved the small intestines in 75% and the large intestines in 25% of the cases. Sixty-nine percent of all lacerations were minor, and 31% were major. Seventy-two percent of the lacerations occurred during uncomplicated gynecologic operations. All lacerations were repaired with one or more of four basic procedures: one- or two-layer closure of intestinal lacerations, partial bowel resection, intestinal reanastomosis, and colostomy. It is suggested that gynecologists acquire basic knowledge in the prevention of intestinal injury and the principles of repair of intestinal lacerations. The role of animal surgical laboratories for the training of residents practicing gynecologic surgery is emphasized. (*AM J OBSTET GYNECOL* 1986;155:509-14.)

Key words: Intestinal injury, gynecologic surgery, bowel complications

The proximity of portions of the intestinal tract to the internal female genitalia, as well as the common involvement of both organ systems in endometriosis, inflammatory disease, or carcinoma, predisposes the bowel to injury during gynecologic surgery. However, residents in obstetrics and gynecology are not routinely trained to perform intestinal operations. A problem in teaching the principles of prevention and management of intestinal lacerations is the lack of information in the literature. For this reason, I investigated the frequency, cause, and treatment of intestinal injury incidental to gynecologic operations during a 10-year period at the Medical College of Virginia. Derived from this experience are guidelines for teaching trainees in gynecology the principles of prevention and management of intestinal injury.

Material and methods

I conducted a computer-aided search of approximately 17,650 records of patients who had undergone gynecologic operations at the Medical College of Virginia from 1973 to 1982. A total of 128 cases of intestinal injury were coded as "complication" on the discharge summary sheet or could be retrieved from operating room records. The charts of these patients were then studied with emphasis on operative reports and postoperative progress notes.

Injury was classified as "superficial" when it involved the serosal or superficial muscular layer of the intestine. "Minor injury" penetrates the mucosa of the bowel in circumscribed areas, whereas "major injury" interrupts the intestinal mucosa in multiple sites or large areas or transects the bowel completely. Superficial injuries were excluded from the study as they appeared to be recorded inconsistently in charts and were infrequently identified as complications in operative notes or discharge summaries.

p Values <0.05 were considered significant. Calculation of statistical significance was by the χ^2 test in all cases.

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Table I. Incidence of intestinal injury occurring during entrance of the peritoneal cavity for various indications

Indications for laparotomy*	Total No.†	Intestinal injury‡	
		No.	%
Uterine disease	3440	28	0.8
Neoplasms, benign	2840	22	0.7
Neoplasms, malignant	480	5	1.0
Miscellaneous	120	1	0.8
Adnexal disease	1970	17	0.9
Neoplasms, benign	930	8	0.9
Neoplasms, malignant	340	5	1.5
Infectious	150	2	1.3
Miscellaneous§	550	2	0.4
Endometriosis	160	2	1.3
Miscellaneous	130	1	0.8
Total	5700	48	0.8

*May include primary operations (exploration and resection) as well as secondary surgical procedures (reexploration, treatment of complications).

†Approximate figures.

‡All cases involved the small intestines.

§Mostly tubal operations for infertility.

Febrile morbidity was defined as two temperature elevations of $\geq 100.4^{\circ}$ F on two separate occasions at least 6 hours apart.

Results

During the 10-year period from 1973 to 1982, a total of 128 incidences of bowel injury were recorded in the charts of approximately 17,650 patients undergoing gynecologic surgery at the Medical College of Virginia. Of all lacerations, 48 (37.5%) occurred during entrance into the peritoneal cavity, 45 (35.2%) during lysis of adhesions or performance of pelvic or abdominal dissections, 13 (10.2%) during laparoscopy, 11 (8.6%) during vaginal operations and 11 (8.6%) during dilatation and curettage and dilatation and evacuation.

The incidence of intestinal injury occurring during entrance into the peritoneal cavity for various indications is shown in Table I. All injuries involved the small intestines. No significant difference regarding the incidences of bowel injury between various groups of indications was noted ($p > 0.05$). More than half (27 of 48, 56.3%) of all bowel lacerations were in women with previous laparotomies (1650 cases). The incidence of intestinal injury (1.6%) in this group was significantly higher than in women who had no previous abdominal operations (21 bowel lacerations in 4050 cases, 0.5%; $p < 0.001$). In the latter group, there were 780 women weighing more than 200 pounds (91 kg). Eight of these patients (1%) suffered bowel injury. By contrast, only 13 of 3270 women weighing ≤ 200 pounds (0.4%) had

bowel lacerations. The difference is statistically highly significant ($p < 0.001$) and suggests that obesity is an independent risk factor for bowel injury. The distribution of indications for laparotomy shown in Table I was similar for patients with or without prior laparotomy, whether obese or nonobese ($p > 0.05$).

Table II provides detailed information regarding the incidence of injury to the small and large intestines occurring during lysis of adhesions or performance of pelvic or abdominal dissections for various indications. Operations for endometriosis were associated with a higher rate of intestinal injury than operations for uterine or adnexal disease ($p < 0.05$). There was no significant difference, however, when the incidence of intestinal injury during operation for endometriosis was compared with the incidence of bowel lacerations associated with surgical treatment of malignant uterine and malignant or infectious adnexal disease ($p > 0.05$). Injury occurring during lysis of adhesions or performance of pelvic or abdominal dissections involved the small intestines in 60% and the large intestines in 40% of the cases.

Combining injury from entering the abdominal cavity with injury from lysis of adhesions or pelvic or abdominal dissections resulted in a bowel injury rate of 1.6% for all laparotomies.

The incidence of bowel injury associated with laparoscopy was 13 in 3710 (0.4%). A total of 2650 of the procedures (71%) were for tubal sterilizations. Six patients in this group (0.2%) suffered injuries to the small intestines. Four of these were trocar injuries, and two were thermal injuries secondary to unipolar electrocoagulation. Two patients (0.1%) had injury to the large intestine resulting from trocar perforation of the transverse colon. Tubal sterilization by electrocoagulation was replaced by other methods (silicone elastomer rings, clips) in our institution in 1976. Of the 1060 diagnostic laparoscopies, five (0.5%) resulted in injury to the small and large intestines (four cases and one case, respectively) during insertion of the trocar. All eight cases of trocar injuries to the small intestines occurred in women with the small bowel adherent to the anterior abdominal wall. All of these women had previous abdominal operations.

Vaginal operation led to bowel injury in 11 of 965 cases (1.1%) and occurred most commonly during opening of the posterior cul-de-sac as part of vaginal hysterectomy (three of 560 cases, 0.5%), culdoscopy (two of 110 cases, 1.8%), transvaginal tubal sterilization (two of 90 cases, 2.2%), and transvaginal drainage of a pelvic abscess (two of 45 cases, 4.4%). Six of the injuries involved the rectum or sigmoid colon; in three cases the small bowel was involved. Two of 160 patients suffered perforation of the rectum during posterior colporrhaphy (1.3%). Since 1974, transvaginal sterilization

Table II. Incidence of intestinal injury occurring during lysis of adhesions or performance of pelvic or abdominal dissections for various indications

Indications for laparotomy*	Total No.†	Intestinal injury					
		Small bowel		Large bowel		• Total	
		No.	%	No.	%	No.	%
Uterine disease	3440	11	0.3	6	0.2	17	0.5
Neoplasms, benign	2840	5	0.2	3	0.1	8	0.3
Neoplasms, malignant	480	5	1.0	3	0.6	8	1.7
Miscellaneous	120	1	0.8	0	0	1	0.8
Adnexal disease	1450	14	1.0	8	0.6	22	1.5
Neoplasms, benign	930	3	0.3	1	0.1	4	0.4
Neoplasms, malignant	340	7	2.1	5	1.5	12	3.5
Infectious	150	3	2.0	2	1.3	5	3.3
Miscellaneous‡	550	1	0.2	0	0	1	0.2
Endometriosis	160	2	1.3	4	2.5	6	3.8
Miscellaneous	130	0	0	0	0	0	0
Total	5700	27	0.4	18	0.3	45	0.8

*May include primary and secondary surgical procedures.

†Approximate figures.

‡Mostly tubal operations for infertility.

and culdoscopy have rarely been performed at the Medical College of Virginia.

Dilatation and curettage (565 cases) resulted in one (0.2%) bowel laceration and dilatation and evacuation for the purpose of therapeutic abortion or incomplete abortion (7,010 cases) resulted in 10 (0.1%). In seven cases the small intestines were involved; in four cases the large intestines were involved.

Minor lacerations accounted for 68.8% of all intestinal injuries, and 31.2% of lacerations were major. Of the 96 small bowel lacerations 65 (67.7%) were minor and 31 (32.3%) were major. A similar relationship was found for the 32 colorectal injuries: 23 (71.9%) lacerations were minor and nine (28.1%) were major. The number of minor lacerations was twice the number of major lacerations occurring in the laparotomy, laparoscopy, and vaginal operation groups. In the dilatation and curettage and dilatation and evacuation groups, however, there was an inverse relationship with major lacerations accounting for eight (72.7%) of the 11 cases.

If obesity and prior operation are not counted as risk factors, then 36 (28%) of the 128 patients were at risk for a bowel injury as there were extensive adhesions or involvement of bowel by neoplasms, infections, or endometriosis. Seventy-two percent of the lacerations occurred during "uncomplicated" gynecologic operations.

All lacerations were repaired with the use of one or more of four basic procedures: one- or two-layer closure of intestinal lacerations, partial small bowel resection, intestinal reanastomosis, and colostomy (Table III). None of the intestinal injuries detected and managed appropriately during the operations resulted in

Table III. Repair of intestinal injury

Injury	Repair	No.
Small intestines		
Minor injury	One- or two-layer closure	62
Major injury	One- or two-layer closure	6
	Reanastomosis	13
	Partial resection and reanastomosis	15
Large intestines		
Minor injury	One- or two-layer closure	20
Major injury	One- or two-layer closure	2
	Reanastomosis	1
	Partial resection and reanastomosis	2
	Partial resection, reanastomosis, and proximal colostomy	4
	Partial resection, Hartmann procedure, and proximal colostomy	3
Total		128

serious long-term sequelae. The postoperative complications are described in Table IV.

Comment

The incidence of intestinal tract injury incidental to gynecologic operations is difficult to estimate as superficial injury is rarely documented. During the 10-year period from 1973 to 1982, a total of 128 (0.7%) cases of bowel injury were recorded in the charts of approximately 17,650 patients undergoing gynecologic operations at the Medical College of Virginia. Of all lacerations, 37% occurred during entrance into the peritoneal cavity, 35% during lysis of adhesions or per-

Table IV. Postoperative complications*

<i>Complication</i>	<i>No.</i>
Wound infection	7
Atelectasis, pneumonia	6
Febrile morbidity (not specified)	6
Urinary tract infection	3
Ileus	4
Peritonitis	2
Anastomotic leak	1
Other†	2

*Five patients had more than one complication.

†Myocardial infarction, gastrointestinal bleeding.

formance of pelvic or abdominal dissections, 10% during laparoscopy, 9% during vaginal operations, and 9% during dilatation and curettage and dilatation and evacuation.

If obesity and prior operation are not considered to be risk factors, then 36 (28%) of the 128 patients were at risk for bowel injury as there were extensive adhesions or bowel involvement by neoplasms, infections, or endometriosis. Seventy-two percent of the lacerations, however, occurred during "uncomplicated" gynecologic operations and in retrospect may have been preventable had the proper precautions been taken.

A surprisingly high incidence of injury to the bowel occurred during entrance into the peritoneal cavity (0.8% of 5700 cases). Patients with previous laparotomy had a three times higher risk of intestinal injury on entrance into the peritoneal cavity than patients who had no previous abdominal operations. This relationship was independent from the indication for laparotomy. Safeguards for entering the peritoneal cavity directly through an old abdominal incision would include extending the new incision above the old one and entering the peritoneal cavity in the superior part of the incision.¹

Among patients with no previous abdominal operations, obese women appeared to be at higher risk for bowel injury than nonobese women. In the present series, the incidence of bowel injury during entry into the abdominal cavity was two and a half times higher in the obese patient as compared with the patient of normal weight. In several instances the small bowel was cut when it was grasped by forceps in the attempt to elevate the peritoneum. This can be prevented by dissecting the preperitoneal fat down to the peritoneum, grasping and tenting the peritoneum with smooth forceps, rolling it between the thumb and index finger, and then regrasping it with forceps.¹

Intestinal injury occurring during lysis of adhesions or performance of pelvic or abdominal dissections formed the second largest group in my series. In this group of patients a close relationship with the under-

lying disease process was observed. It is therefore not surprising that patients with a malignant neoplasm, an infectious disease process, or endometriosis suffered a significantly higher incidence of bowel injury than patients with other indications for laparotomy (Table II). Some bowel lacerations apparently occurred during blunt separation of loops of bowel. While blunt dissection is appropriate in cases of filmy adhesions, sharp dissection is preferred when the adhesions are dense or the tissue planes destroyed.² The tissue should be kept under tension during dissection and an attempt should be made to dissect along anatomic planes.

The incidence of bowel injury associated with laparoscopy was 13 (0.4%) in 3710. Thermal injury in this group was only observed in two operations that were performed during the first four years of the series. After 1976, silicone elastomer bands or clips were used for tubal sterilizations and no more thermal bowel damage was documented. Trocar injury to the large bowel occurred in three cases. This could have been avoided by nasogastric suction to deflate the stomach or by routine tracheal intubation in all cases of general anesthesia.³ All eight cases of trocar injuries to the small intestines occurred in women with small bowel adherent to the anterior abdominal wall. All had previous abdominal operations. Inserting the trocar outside of an old incision or using the open laparoscopy technique will help to avoid complications when adhesions in the area of an old incision are suspected.⁴

Vaginal operations led to bowel injury in 11 (1.1%) of 965 cases in the present series. Since elimination of culdoscopy and transvaginal tubal sterilization at my institution, the incidence of bowel complications associated with vaginal operations has declined. Bowel injuries during routine vaginal hysterectomy or posterior colporrhaphy may be reflections of faulty surgical technique⁵ but may also be secondary to scar tissue formation with loss of tissue planes or fixation of bowel loops within the posterior cul-de-sac from adhesions, tumor, or endometriosis.

Dilatation and evacuation procedures for termination of pregnancy were associated with bowel injury in 0.1% of cases. In eight of the cases, however, the bowel damage was severe. By contrast, bowel injury associated with laparotomy, dissections, lysis of adhesions, laparoscopy, and vaginal operations was major in only one third of the cases.

All lacerations were repaired with the use of one or more of four basic procedures: one- or two-layer closure of intestinal lacerations, partial bowel resection, intestinal reanastomosis, and colostomy (Table III). One- or two-layer closure was the only means of repair in 70% of all patients. This procedure is easily taught and should be well mastered by residents completing their training in obstetrics and gynecology. Partial small

bowel resection, intestinal reanastomosis, and colostomy, however, are usually viewed as difficult surgical procedures beyond the scope of residency training in obstetrics and gynecology.

During recent years, residents in obstetrics and gynecology at the Medical College of Virginia have been instructed in animal surgery laboratories to repair minor and major bowel lacerations. In my opinion, residents have become more confident in handling the bowel and will perform colostomies with little difficulty. Some residents with sufficient exposure to bowel surgery in humans may learn to perform segmental resection and reanastomosis for repair of major lacerations. Most notably the Gambee single-layer open anastomosis⁶ but also the side-to-side anastomosis with the use of automatic stapling devices⁷ have removed the complexity from intestinal anastomosis. Both procedures are technically simple but above all very effective and safe means of bowel anastomosis.⁷⁻⁹ They are therefore eminently suitable to be taught in residency training programs.

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Discussion

DR. WILLIAM DROEGEMUELLER, Chapel Hill, North Carolina (Official Guest). This article will be a valuable addition to our literature for two reasons: first because it is a review with many excellent teaching points and second because it establishes the numerical frequency of intestinal injury related to gynecologic surgery.

It is overwhelmingly difficult if not impossible to perform a randomized and controlled study of an extremely rare complication of elective surgery. Thus

painstaking record reviews continue to be our only insight into the frequency and cause of rare surgical complications. Without data such as those presented in this article, often the surgical misfortune, for example, excessive bleeding during a circumcision, is blamed on the experience of the surgeon. If a student performs the circumcision, the bleeding is due to his or her lack of experience. If a resident is the surgeon, the rationale is that the resident performs many circumcisions and a rare one bleeds. If the department chairman performs a circumcision and it bleeds, we perform a workup of the newborn infant for hemophilia. Purists would find fault with several of Dr. Krebs' conclusions regarding cause and management for he does not present enough data to justify his conclusions. To be fair, Dr. Krebs preceded one section by stating "In my opinion." Thus he appreciates that the article does reflect his own bias.

Let us review the major teaching points of this article. Approximately one half of intestinal injuries associated with major abdominal surgery occur while the peritoneal cavity is entered. Two groups are at a threefold higher risk, the obese and women with previous abdominal incisions. The remaining intestinal injuries occur during lysis of adhesions and while abdominal and pelvic dissections are performed. Not surprisingly, women with endometriosis, pelvic infection, or carcinoma were at highest risk. I have no quarrel with the time-honored suggestions in surgical technique that Dr. Krebs emphasizes in this paper: the use of sharp dissection in dense adhesions, an open laparoscopy technique with adhesive disease, and entry into the peritoneal cavity by a gentle technique superior to the extent of previous incisions.

The key question is how many of the bowel injuries were preventable. On this question, I differ from Dr. Krebs. I believe that the surgical principles elucidated in this paper are well known. It is not a lack of knowledge that lead to intestinal injury. Only impatience during the surgical procedure would cause a surgeon to deviate from standard teaching. Thus I would speculate that the vast majority of intestinal injuries are not preventable. Last, there is one sentence in the paper that would be a lawyer's fantasy and a gynecologist's nightmare: "Bowel injuries during posterior colporrhaphy are usually reflections of faulty surgical technique." I do not agree with this basic premise.

The article underlines the importance of animal surgical experience in residency training. I believe the expense of an animal laboratory is compensated by the valuable learning experiences it presents to our residents, although we have noted over the years that dogs seldom have the extensive dense adhesions encountered in clinical gynecology.

To stimulate discussion, I would like Dr. Krebs to comment on injury to bowel that has been previously irradiated. Damage to irradiated bowel is a special category of injury on which he did not comment.

In summary, this article documents the frequency of intestinal injury related to gynecologic surgery. Since

the article reflects a 10-year experience from a major university hospital, with the chairman of this department being internationally recognized as an innovator of gynecologic surgical technique and the department having an excellent active gynecologic oncology division, I doubt the injuries were due to lack of knowledge. The vast majority of bowel injuries are unavoidable.

DR. PAUL UNDERWOOD, Charlottesville, Virginia. I agree with you that the gynecologist should be taught how to repair bowel injuries as well as urinary tract injuries. I thoroughly enjoyed your presentation. One question that you did not answer was how many of these intestinal injuries were repaired by the gynecologic service and how many of them were repaired by the surgical service?

DR. JAROSLAV HULKA, Chapel Hill, North Carolina. A good deal of electrocoagulation is being used, not for sterilization but for control of bleeding. This brings up the very real possibility of electrocoagulation injury during gynecologic surgery and I would like to mention to this group the findings of Dr. Wheelless, of The Johns Hopkins University School of Medicine, a few years ago, of the management of this complication. The unipolar injury looks small but the mucosa is quite sensitive to this kind of injury. The repair of a small injury involves extensive dissection and anastomosis because about 4 or 5 cm of the small bowel is usually destroyed beyond the small white discoloration of the electrocoagulation site. Those of us who use bipolar coagulation do not have this problem because with bipolar "what you see is what you get." There is no extension of the thermal injury because of the electron-seeking base currents. Therefore, I would like to urge that people using electrocoagulation, which is a fine means of controlling bleeding, keep in mind the important difference between unipolar and bipolar injuries and their managements.

DR. EDWARD JAMES DENNIS III, Columbia, South Carolina. Between the years 1972 and 1976, I was a one-man department in using radiation therapy and treatment in a cancer ward. I can assure you I resected or bypassed more of the small bowel than any of the general surgeons in the Columbia area. There is one area, which Dr. Underwood has already addressed, that we, as gynecologist-oncologists, particularly when we are dealing with an irradiated bowel, should not call in a general surgeon to help us because this would be doomed to failure. They do not understand the damage that is done to the small bowel by megavoltage, full-blown irradiation to the pelvis. I know how to use sta-

ples but I do not believe that stapling procedures are indicated in bowel that has been irradiated. I do think, and it has already been alluded to, that the gynecologic oncology service is a great time to teach residents how to do the bowel anastomosis, which is a very simple procedure. Surgeons would like to think that you have to have a special gift from above to do this, but, as it was just pointed out, there are certain basic principles that one must adhere to, not only in the actual technique of the operation but in the preoperative and postoperative management of the patient.

DR. KREBS (Closing). Dr. Dennis points out that gynecologists should handle bowel injury associated with radiation therapy. I agree, but a general surgeon would be the better one to handle such cases if the gynecologist is not experienced in bowel surgery. The worst situation to gain experience with bowel surgery is with irradiated bowel. This should be handled primarily by the gynecologic oncologist. In regard to the stapling techniques, there are diverse opinions about the usage of staples. Most gynecologic oncologist will probably argue that the stapling technique decreases the blood flow in the anastomosis less than hand-sewn anastomosis.

Dr. Underwood asks who repairs the injured bowel. In the cases presented here, possibly with a few exceptions, the injuries were repaired by gynecologists. Our general gynecologists are capable of performing the major types of bowel operations I mentioned. We do have three people in our department who are trained in bowel surgery, including the chairman and two oncologists.

Dr. Hulka's point in regard to electrocoagulation is well taken. We have not observed any further bowel laceration since electrocoagulation for the purpose of tubal sterilization was discontinued. If used for coagulation of bleeders or endometriotic implants, bipolar techniques are preferred over unipolar ones.

Finally, Dr. Droegemueller suggests that I alter the statement, "Bowel injuries during posterior colporrhaphy are usually reflections of faulty surgical technique." I concur, as the bowel is sometimes densely adherent to the cul-de-sac and it is almost unavoidable to enter the bowel in an attempt to open the cul-de-sac. Also, the term preventable bowel injury ought to be used with caution. Even experienced surgeons who have taken proper precautions have entered the bowel inadvertently. This article has established that obesity and prior laparotomy are risk factors for bowel injury and should be added to the 28% of risk factors related to extensive adhesions, neoplasms, infections, or endometriosis.

CA 125 in gynecologic practice

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Serum CA 125 levels were determined in 64 women with benign ovarian lesions, 92 women with uterine fundal lesions, and six patients who had negative second-look laparotomy for epithelial ovarian carcinoma. Of those with benign lesions, 13 of 31 patients with endometriosis had levels >35 U/ml. Six of 34 patients with endometrial carcinoma had elevated levels before the primary operation, and six of 15 patients with recurrent endometrial carcinoma had elevated levels. The six ovarian cancer patients had had negative findings at second look 7 to 40 months before recurrence. Where close serial levels were available, the level became elevated 2 to 5 months before clinically apparent recurrent disease was noted. (AM J OBSTET GYNECOL 1986;155:515-8.)

Key words: Murine monoclonal antibody, endometriosis, endometrial adenocarcinoma

In 1975 Kohler and Milstein¹ produced monoclonal immunoglobulins using somatic cell hybridization. In 1981 Bast et al.² described the production of a murine monoclonal antibody termed OC 125 with specificity for some nonmucinous human ovarian carcinomas. This murine monoclonal antibody was developed by somatic hybridization of spleen cells of mice immunized with an epithelial cell line OVCA433 obtained from a patient with papillary cystadenocarcinoma. It was noted to be an immunoglobulin G1 and it was immunologically dissimilar from carcinoembryonic antigen. The murine monoclonal antibody reacted with all six ovarian cancer cell lines tested but with only one (a melanoma) of 14 other cancer cell lines examined. The antigenic determinant against which OC 125 is directed has been termed CA 125.

Kabawat et al.,³ using sections of ovarian tumors obtained at operation, incubated these sections with OC 125 antibody. Of the 60 ovarian tumors screened, 34 (56.6%) bound to the antibody by the immunofluorescent technique used. Five of these reactive tumors were as follows: benign papillary serous cystadenoma, one; serous cystadenoma, one; and papillary serous cystadenofibroma, three. Of the malignant lesions, 19 of 23 papillary serous cystadenocarcinomas, two of two

mixed papillary serous and endometrioid adenocarcinomas, two of three endometrioid adenocarcinomas, one of four clear cell carcinomas, two of two undifferentiated carcinomas, and two of two borderline serous cystadenocarcinomas were reactive. No reactivity was seen in the eight mucinous epithelial lesions, in the five sex cord tumors, or in the three germ cell tumors tested. These authors observed that the staining, when antigen positive, was on the cell surface and not in the cytoplasm. In cystic, glandular, or papillary tumors, staining could be observed on the luminal surface of the epithelium. The reactivity did not relate to the grade of the tumor. Also metastatic lesions reacted in the same manner as the tumor from the primary site. There was heterogeneity within any given tumor. The authors suggested that this technique might be used to identify malignant cells of ovarian origin when cells of uncertain origin were found in ascitic fluid.

Klug et al.,⁴ using a CA 125 immunoradiometric assay and a normal mixed population, found that there were decreasing CA 125 serum levels with increasing age in both sexes. Also the levels were greater in women than in men.

Niloff et al.⁵ determined CA 125 levels in 988 women with benign gynecologic disorders. They observed the CA 125 level to be >65 U/ml in 1% and in 0.5%, respectively, of those studied with two determinations; these samples were drawn at different times. They further determined that 15.8% of women in the first trimester of pregnancy had levels >65 U/ml but no woman in the latter two trimesters had positive serum levels. Levels were markedly elevated in amniotic fluid from all trimesters. All of the positive CA 125 levels associated with entities other than ovarian malignancy were found in women <50 years of age. In women over 40 years of age who would be at greatest risk for ovarian

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Table I. CA 125 in clinically palpable benign ovarian tumors

Disease	CA 125		No. positive* per total no.
	Range	Mean	
Endometriosis			
Stage I	12-54	30.2	2/4
Stage II	8-83	27.6	2/9
Stage III	6.2-726	—	6/13
Stage IV	30.5-102	52.8	3/5
Benign teratoma	6-25	14.2	0/19
Follicular phase cyst	4.0-81	14.6	1/16
Luteal phase cyst	4.3-162	34.1	2/8

*CA 125 level > 35 U/ml.

Table II. CA 125 in uterine fundal lesions

Tumor	CA 125
1. Sarcomas	
Initial values (n = 6)	All < 35 U/ml
Recurrences and active disease (n = 13)	2 > 35 U/ml 1 rose from 29 to 332; 1 rose from 59 to 237
2. Adenocarcinomas	
Initial (n = 34)	6 > 35 U/ml 60, 2790, 555, 293, 103, 307
Recurrent (n = 15)	6 > 35 U/ml 75, 138, 43, 170, 127, 1552
3. Leiomyomas (n = 24)	5 > 35 U/ml 37, 59, 72, 55, 49

Numbers in parentheses represent the number of patients in each group.

carcinoma, they calculated the specificity of this assay as 99%.

Ricolleau et al.⁶ found 13 of 14 patients with cirrhosis had elevated CA 125 levels. In addition, 21 of 37 (57%) patients with recurrent colon, pancreas, and breast carcinomas had CA 125 levels >30 U/ml. Klug et al.⁴ also reported that 59% of patients with pancreatic cancer, 32% with lung cancer, 31% with other gastrointestinal cancers, 22% with colorectal cancers, and 12% with breast cancers had CA 125 levels >35 U/ml.

Bast et al.⁷ reported significant positive correlation between CA 125 levels and regression, stability, and progression of epithelial ovarian cancers. Decreasing CA 125 levels occurred in 20 of 21 cases with regression of disease. Some of these patients, with levels <35 U/ml, were found at second look to have had multiple implants as large as 1 cm in diameter. Steadily rising values for CA 125 occurred in each of the 17 cases with progression of tumor.

Lavin et al.⁸ defined profiles in ovarian cancer patients undergoing therapy in three categories: (1) patients with complete cytoreductive surgical procedures in whom levels always fell to <35 U/ml within the first 3 months and stayed below that level, (2) patients with

incomplete cytoreductive surgical procedures in whom levels fell to <35 U/ml when they were responding to chemotherapy, and (3) patients in whom serum CA 125 levels never fell to <35 U/ml.

Niloff et al.,⁵ using these categories, reported that patients in category 1 were free of tumor at second look 35% of the time, those in category 2 were free of tumor at second look 10% of the time, and those in category 3 were found to have persistent or progressive disease.

Berek et al.¹⁰ evaluated the CA 125 levels within a week before second look and noted that none of the patients with a negative second look had CA 125 antigen levels $\geq$ 35 U/ml. These authors also noted that when the CA 125 antigen level was $\geq$ 35 U/ml, all patients had tumor at second look.

Niloff et al.¹¹ reported CA 125 levels >35 U/ml in one of seven (14.3%) patients with squamous carcinoma of the vulva, one of 14 (7.1%) patients with squamous carcinoma of the cervix, five of six (83.3%) patients with adenocarcinoma of the cervix, 14 of 29 (48.3%) patients with adenocarcinoma of the endometrium, and four of four patients with carcinoma of the fallopian tube.

In the present study we evaluated CA 125 serum levels among patients with nonmalignant ovarian lesions or uterine fundal lesions and in a small group of patients with ovarian cancer after second-look operation. The results are discussed with respect to the potential value in clinical practice.

Methods

Serum from 64 patients with nonmalignant ovarian lesions and 92 patients with uterine fundal lesions was collected preoperatively. Six patients with malignant ovarian lesions who had previously undergone negative second-look procedures had serial serum samples evaluated.

Pathologic diagnosis was confirmed at the time of definitive operation. Staging for the patients with endometriosis was done with the American Fertility Society classification published in 1979.¹²

The immunoradiometric assay for CA 125 in peripheral blood was performed as detailed in the kit (manufactured at Centocor) and as previously described by Bast et al.⁷ and Klug et al.⁴

Results

Endometriosis. Elevated CA 125 levels (>35 U/ml) were found in 13 of 31 patients with endometriosis (four of 13 with Stage I and II disease and nine of 18 with Stage III and IV disease). Sensitivity and specificity of CA 125 at levels >35 U/ml were calculated under two circumstances: (1) in those patients with endometriosis (31) in the entire group of patients with benign palpable ovarian lesions (64) and (2) in patients with Stage III and IV endometriosis. For these groups, sen-

sitivity and specificity were (1) 29.5% and 91.6% and (2) 33% and 91.6%, respectively.

Nonmalignant ovarian lesions. Benign teratomas produced no elevated CA 125 levels in nine patients studied. One of 16 patients with follicular phase cysts had an elevated level as did two of eight patients with luteal phase cysts (Table I).

Uterine fundal lesions. CA 125 levels >35 U/ml were detected in two of 13 patients with recurrent or active uterine sarcomas, one with carcinosarcoma, and one with leiomyosarcoma. Six of 34 patients with adenocarcinoma of the endometrium and six of 15 patients with recurrent endometrial carcinoma had elevated CA 125 levels before operation. There were no elevated levels in six patients with primary untreated uterine sarcomas. Five of 24 patients with leiomyomas had elevated CA 125 values; however, these were elevated to a minimal degree (Table II).

Results after second look. Table III shows that the elapsed time from negative second look to recurrence in 6 patients ranged from 7 to 40 months. In the patients with the long relapse-free intervals, there may have been recurrence earlier than the test or examination would indicate since they were being evaluated at 6-month intervals. In three cases, the CA 125 level was elevated 2 to 5 months before progressive recurrence was recognized. Two of these patients had levels that fell briefly in response to chemotherapy.

Comment

Bast et al.⁷ found that less than 1% of the normal population had CA 125 levels >35 U/ml. In our study group, patients with known gynecologic problems were selected. With the CA 125 assay, elevated levels were found in 15 of 64 patients with benign ovarian lesions, of which 13 of the 15 positive values occurred in 31 patients with endometriosis. These levels were considered elevated if results were >35 U/ml. In their study, Niloff et al.⁵ used 65 U/ml and found no positive results in 35 such patients, 10 of whom had endometriosis.

Although the serum CA 125 levels in young patients with palpable ovarian lesions are usually not elevated, the fact that elevations occur in a significant fraction of women with advanced endometriosis suggests that diagnostic application of the test may be possible in this population. For example, Barbieri et al.,¹³ obtaining similar results among a group of endometriosis patients, have suggested that patients with unexplained infertility and elevated CA 125 values might have a diagnostic laparoscopy schedule early in their workup.

The relatively low serum CA 125 levels (<65 U/ml) among most patients with benign masses compared with the elevated levels in ovarian cancer patients reported by Bast et al.⁷ also suggest that the serum CA 125 level may be a helpful diagnostic adjunct in eval-

Table III. Interval between first elevated CA 125 level and clinical recognition of progression of disease in patients with relapse after negative second look

Patient No.	Date of negative second look	Date of positive CA 125	Date recurrence recognized clinically
1	April, 1980	October, 1983	October, 1983
2	November, 1982	February, 1984	June, 1984
3	July, 1981	October, 1984	October, 1984
4	November, 1981	April, 1983	April, 1983
5	September, 1981	February, 1983	April, 1983
6	May, 1983	January, 1984	June, 1984

uating patients for malignancy. Einhorn et al.¹⁴ have recently proposed this possibility as well, as a result of a study of serum CA 125 levels of 100 women with pelvic masses.

Niloff et al.¹¹ reported that 48% of patients with endometrial carcinomas had elevated serum CA 125 levels. None of those with early stage disease had elevated levels in this study. Consistent with this result in our series, serum CA 125 elevations were found in only 17.6% of patients at the time of their initial treatment and in 40.0% with clinically identified recurrent and metastatic disease. Although only a relatively small fraction have positive levels, it may be worthwhile to use alterations in these levels to determine the patients' progress. A large study would be required to verify this hypothesis. It is of interest, but of no clinical usefulness, that two of 13 patients with recurrent sarcomas had a positive level.

Although several authors have addressed the use of CA 125 levels before operation, during primary therapy, and until second-look laparotomy, the five cases noted here, all with initial negative second-look operations, would suggest that CA 125 levels after second look are appropriate and should carry significant import when they become elevated, even though clinical signs have not yet appeared. Podratz et al.¹⁵ evaluated recurrences in 77 patients with negative second-look laparotomies for ovarian cancer. There were recurrences detected in 12 patients between 6 and 41 months after operation. Five of these were in the first year and four more in the second year. With these findings, it might be considered appropriate to determine CA 125 levels monthly in the first year, every 2 months in the second year, and every 3 to 6 months thereafter in evaluating patients for recurrence of disease after second look procedures. Other preliminary studies by us suggest that any patients, male or female, having abdominal operations for benign or malignant disease may have a transient positive postoperative level of CA

125 lasting for a month or two. Consequently, positive levels in the immediate postoperative period require cautious evaluation and repeat determinations.

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Extramammary Paget's disease of the vulva and anus: Use of intraoperative frozen-section margins

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Thirteen consecutive cases of vulvar Paget's disease treated by us between 1975 and 1984 underwent pathologic retrospective review. In the first group of eight patients having Paget's disease not involving the anal mucosa, the extent of disease was completely defined by frozen-section margins. Additional intraoperative resections were necessary in five of the eight. None had residual involvement on permanent sections and none had recurrences in 3 to 8 years of follow-up, although two died of unrelated causes. A second group of two patients early in the series had frozen sections to define some but not all of the margins. Both had positive perineal margins on permanent sections. One has required two subsequent revisions after 3 years of follow-up and the other has been free of disease during 9 years of follow-up. A third group of three patients all had anal mucosal involvement and all had associated mucinous adenocarcinoma of the rectum. Frozen sections actually discovered the presence of one of the three carcinomas. After appropriate radical operations, all three are alive and free of disease between 2 and 9 years of follow-up. It is concluded: (1) Patients with vulvar Paget's disease can reduce the need for subsequent operations with the use of frozen sections to define surgical margins; (2) anal mucosal involvement of Paget's disease represents an ominous sign of underlying carcinoma. (AM J OBSTET GYNECOL 1986;155:519-23.)

Key words: Paget's disease of vulva, anus, intraoperative frozen section

Extramammary Paget's disease of the vulva is a pruritic, erythematous, or scaly neoplasm of the elderly, first described by Crocker¹ in 1889 and given that name because of the lesion's resemblance to the breast lesions described by Paget in 1874. As emphasized by Jones et al. (2) the lesion may be: (1) totally confined to the epidermis,² locally invasive, or (3) associated with a sub-jacent adenocarcinoma. Distant second malignancies can be found in up to 25% of cases.²⁻⁴ Local recurrences because of horizontal spread or because of a multifocal origin has been reported in 35%.² Anal involvement of Paget's disease carries about a 70% risk of a sub-jacent rectal carcinoma.^{2, 5-7}

Beginning in 1979, we instituted the use of intraoperative frozen-section margins to define the extent of disease, an approach not commonly mentioned in the literature. The purpose of this paper is to document the use of this technique in minimizing recurrences, as well as to emphasize the hazard of anal involvement by Paget's disease.

Material and methods

Thirteen consecutive cases of extramammary Paget's disease of the vulva treated at Crawford W. Long and St. Joseph's Hospitals in Atlanta, Georgia, between 1975 and 1984 underwent a retrospective pathologic review. Ten of the 13 were treated by us. Three groups of patients were defined. The first consisted of eight patients treated between 1979 and 1984 in whom all surgical margins were defined by frozen sections. The second group of two patients were those treated before 1979 in who frozen sections were used to define some but not all of the margins. The third group consisted of three patients, all having Paget's disease involving the anal mucosa. Current follow-up information is available in 12 of 13 patients.

A single extended vulvectomy was used for seven of the eight cases in the first group. A radical vulvectomy was used for the remaining patient in whom an underlying carcinoma was suspected. A simple vulvectomy was used for the two patients in the second group. A variety of procedures were used for the three patients in group 3. Case No. 7 was treated with an abdominoperineal resection with the finding of incidental Paget's disease of the anus. In Case No. 12 a simple vulvectomy with a deep resection was performed in the area of a small rectal carcinoma discovered at frozen section. This patient delayed definitive treatment for 2 years before an abdominoperineal resection. In Case

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Table I. Patient characteristics

<i>Group</i>	<i>Case No.</i>	<i>Age at diagnosis (yr)</i>	<i>Location</i>	<i>Past medical history</i>
Group 2	1	68	Vulva	None
	2	68	Vulva	Diverticulitis, atrophic dermatitis, emphysema, breast biopsy for benign disease, cholelithiasis, multiple allergies
Group 1	3	45	Clitoris and vulva	Increased blood pressure, thyroiditis, cholelithiasis
	4	76	Clitoris and vulva	Breast carcinoma, hypertension, congestive heart failure, hysterectomy for dysfunctional bleeding
	5	71	Vulva and perianal skin	Vulvar dystrophy, breast biopsy for benign disease, psychosis
	6	62	Vulva	Thyroiditis, hysterectomy for fibroids, hypertension, degenerative arthritis, cholelithiasis, psychosis
	8	50	Vulva	Multiple skin cancers, dysfunctional uterine bleeding, breast biopsy for benign disease
	9	76	Vulva	Hypothyroidism, hysterectomy for fibroids, hypertensor, rheumatoid arthritis
	10	67	Vulva	Hysterectomy for fibroids
Group 3	11	81	Vulva	None
	7	67	Anal canal and perianal skin	None
	12	80	Anal canal and perianal skin	Hypertension, depression, fracture of left hip
	13	63	Anal canal and perianal skin	Hypertension

No. 13 the patient was undergoing Moh's chemosurgery for perianal Paget's disease when the underlying carcinoma was defined. Shortly thereafter the patient underwent an anodermal pulldown procedure.

The procedure for obtaining frozen sections began with accurate identification of the margins. Either the entire specimen was placed on a piece of paper accurately labeled as to orientation, or 2 to 3 mm strips of skin obtained circumferentially around the lesion were forwarded, again accurately labeled. When the entire specimen was sent, the margins were stained with India ink and subsequently removed as rectangular prisms parallel to the surgical margins. The specimens were then frozen and examined microscopically, a diagram being maintained for identification of the margin and for the purposes of subsequent dictation of the gross examination report. Any close, deep subcutaneous margins were also examined as frozen sections. If, on frozen section, extramammary Paget's disease was detected, a wider margin was requested from the specific sites identified. The process was repeated until all margins were free of neoplasia.

Permanent sections were made from the surgical specimen including the frozen-section margins, the central tumor, and the uninvolved skin.

Results

The summary of the patient population characteristics is presented in Table I. The average ages in the three groups were similar (group 1, 67½ years; group 2, 68 years; and group 3, 70 years). Multiple chronic disease was common. Four in groups 1 and 2 had evidence of some autoimmunity and two of these 10 (20%) had endured preexisting carcinomas at distant sites, one involving multiple cell carcinomas and the other a breast carcinoma. Four of the 10 had hypertension. Three had undergone cholecystectomy and four had had previous hysterectomy for a benign disease. Two of the group 3 patients had had hypertension.

Table II indicates the status of margins on permanent section, the total number of frozen sections and their locations, the number of positive frozen sections, and current follow-up. Five of the eight group 1 patients had positive frozen-section margins. All were reexcised and all remained free of subsequent Paget's disease. In Case No. 4 the patient has had a recurrence of breast carcinoma in the bone 11 years after original radical mastectomy. She has remained free of recurrent vulvar Paget's disease for 6 years. In Case No. 11 the patient died of a myocardial infarction 14 months after the vulvar operation. There had been no evidence of re-

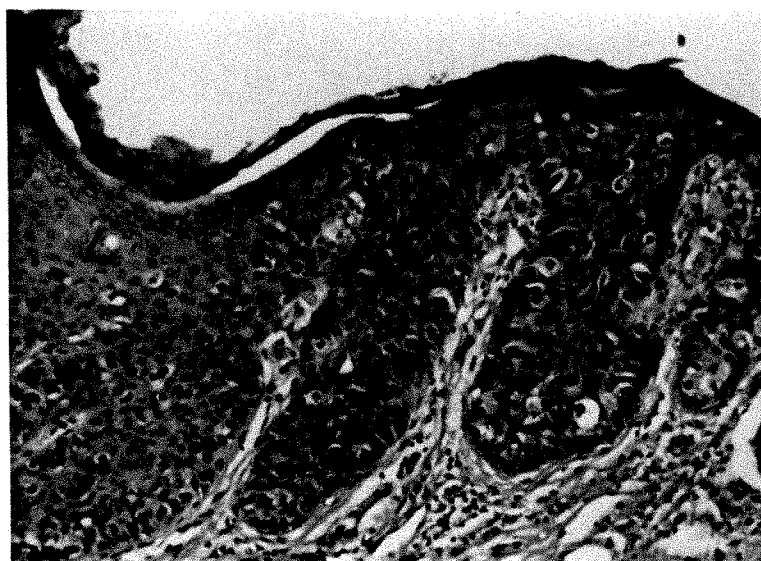


Fig. 1. Paget cells in extramammary Paget's disease with vacuolated cytoplasm. Note the tip of the rete ridge at extreme right of photomicrograph with pseudogland formation. (Original magnification $\times 100$.)

Table II. Vulvar extramammary Paget's disease: pathologic characteristics in groups 1 and 2

Case No.	No. of frozen sections				Number of positive frozen sections	Status of margins on permanent section	Follow-up
	Vaginal margin	Vulvar margin	Perineal margin	Total No.			
1	6	0	0	6	Not enough performed	1 Positive perineal	Two subsequent reexcisions. Patient lost to follow-up after 5 yr
2	6	0	0	6	Not enough performed	1 Positive perineal	NED at 9 yr
3	5	2	0	7	0	Free of disease	NED at 7 1/2 yr
4	0	12	0	12	1 with reexcision	Free of disease	NED for 6 yr; recurrent breast carcinoma in bone after 5 1/2 yr
5	1	3	1	5	0	Free of disease	NED at 5 1/2 yr
6	2	16	0	18	3, All reexcised	Free of disease	NED at 3 yr
8	7	10	0	17	3, All reexcised	Free of disease	NED at 4 yr
9	0	9	0	9	4, All reexcised	Free of disease	NED at 4 yr
10	3	9	4	13	1, Reexcised	Free of disease	NED at 4 yr
11	4	7	0	11	0	Free of disease	Patient died of myocardial infarction at 14 mo

‡NED = No evidence of disease.

currence prior to her death. Both of the patients in group 2 had positive margins for Paget's disease on permanent sections. In Case No. 1 two subsequent excisions have been performed and when last followed up 5 years after initial operation she was free of Paget's disease. In Case No. 2 the patient has been followed up for 9 years without evidence of recurrent Paget's disease.

Table III summarizes further characteristics of group 3. Case No. 12 was actually discovered by frozen section. All three have been treated by the appropriate radical operation although the patient in Case No. 12 delayed an abdominoperineal resection by 2 years and died with disease 1 year after operation.

Comment

James Paget, in his classic article on the mammary areola, also made mention of a condition on the glans penis consistent with extramammary Paget's disease. Paget's disease of the breast and extramammary Paget's disease have in common large cells with round nuclei and pale-staining cytoplasm present among squamous cells of the epidermis (Fig. 1). Whereas Paget's disease of the nipple is always associated with breast adenocarcinoma, extramammary Paget's disease not involving the anal mucosa has only a 20% to 25% association with underlying adenocarcinoma.² Our experience of no subjacent adenocarcinoma in groups 1 and 2 would substantiate the experience of others.²⁻⁴ No locally in-

Table III. Extramammary Paget's disease of the anal canal

Case No.	Initial pathologic findings	Follow-up operation	Final pathologic findings
7	Hemorrhoids and lesion of rectum, 9mm	Abdominoperineal resection	Adenocarcinoma of rectum
12	• Anal and perianal extramammary Paget's disease with segmental resection	Abdominoperineal resection delayed by two yr	Mucin-producing adenocarcinoma of rectum
13	Anal and perianal extramammary Paget's disease with Moh's chemotherapy	Anodermal pull-through	Mucin-producing adenocarcinoma of rectum

vative or metastatic extramammary Paget's disease was found in any of the three groups. The major point to be made is that, in contrasting group 1 with group 2, a willingness to proceed with multiple frozen sections and to resect tissue when required can ensure that such patients will not require subsequent anesthesia and operation for extramammary Paget's disease. In our experience, one could reliably predict that the permanent sections would demonstrate negative margins for Paget's disease once all frozen sections were negative. Our finding is slightly at variance with the findings of Gunn and Gallagher⁹ who demonstrated multiple foci of disease in four patients. An ability to obviate subsequent vulvar operation at the cost of between seven and 18 frozen sections is certainly cost effective.

The origin of the Paget's cell remains controversial. The use of carcinoembryonic antigen¹⁰ and AK4 anti-human cytokeratin antibodies¹¹ would indicate that the Paget's cell is of glandular origin, be that from an adnexal structure or through differentiation of the pluripotential basal cell. The 100% incidence of an underlying rectal adenocarcinoma when the anal mucosa is involved would suggest to us, as it has to others,^{2,6} that Paget's cells may in fact be arising from underlying adenocarcinoma. Both Paget's cells and adenocarcinoma cells stain positively for periodic acid-Schiff in this situation. Our findings in group 3 most certainly emphasize the ominous prognosis for patients having anal involvement of Paget's disease.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. HARRY V. MAY, Greenville, South Carolina (Official Guest). In 1975, Taylor et al.¹ reported on Paget's disease of the vulva. In their retrospective analysis, 15 patients underwent surgical excision in an attempt at definitive therapy. Six patients or 40% had negative surgical margins at the time of permanent sections. All of these patients remained free of recurrence after long-term follow-up. The remaining nine patients or 60% had positive surgical margins at the time of permanent sections. All of these patients had recurrence after an average interval of 45 months.

In an attempt to eliminate the problem of recurrence in Paget's disease of the vulva, Drs. Stacy, Burrell, and Franklin have used frozen sections to accurately define the extent of disease.

The two patients in their group 2 demonstrate the need for complete analysis of the margins by frozen sections. Both patients had positive margins at the time of permanent sections. One required two reexcisions; the other is apparently free of disease after 9 years.

In their group 1, eight patients had totally negative surgical margins at the end of the procedure, as determined by the multiple frozen-section technique. All of these patients were free of recurrence with follow-up of 3 to 7½ years.

There were no cases of subjacent carcinoma in the 10 patients of their groups 1 and 2. This would appear to be in contrast to results in other reports. Lee et al.² found an adenocarcinoma in situ or a frankly invasive adenocarcinoma in 54% of their 13 cases. A 22% incidence of atypia or carcinoma other than Paget's cells

has been reported in another study.¹ Merot et al.³ cited a 33% incidence of underlying carcinoma in nonperianal extramammary Paget's disease of the vulva.

Group 3 in the present study consisted of those three patients with anal mucosal involvement. They all were found to have an underlying rectal mucinous adenocarcinoma. After appropriate surgical intervention, they were all free of disease 2 to 9 years later. The high incidence of underlying adnexal or visceral carcinoma in this class of patients has been confirmed by others.

The histogenesis of extramammary Paget's disease is still controversial. A newly discovered marker for apocrine epithelium—gross cystic disease fluid protein—has been shown to be immunoreactive to Paget's cells in both the perineal and perianal regions.³ These findings suggest an apocrine gland origin or an apocrine differentiation from a multipotential epidermal germ cell. It would seem that gross cystic disease fluid protein and other markers such as carcinoembryonic antigen may eventually lead to a better understanding of the origin of Paget's cells.

In the present article, the incidence of Paget's disease of the vulva relative to the incidence of vulvar carcinoma unassociated with Paget's disease is not stated. I would like to know if the 1% to 2% incidence of previous reports is confirmed by this series of patients.

To conclude, the authors have demonstrated the effectiveness of frozen sections in the treatment of extramammary Paget's disease of the vulva. Of the eight patients in their group 2, five or 63% would have had positive surgical margins at the time of permanent sections if frozen sections had not been used. Some if not all of these patients would have required additional reexcision. They also demonstrated that when the anal mucosa is involved, the risk of underlying carcinoma is extremely high.

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DR. PAUL B. UNDERWOOD, JR., Charlottesville, Virginia. I am somewhat troubled to discuss this article for I believe it is the third one I have reviewed from this outstanding and productive group of gynecologic oncologists. I would not like to be thought of as one singling out their work but, if I were guilty, it would be for excellence and not criticism. This article, like the others, adds a major contribution to our literature.

Paget's disease of the vulva is a rare malignancy. This report of eight cases in the last 5 years would not indicate its uncommon occurrence; however, it probably reflects these physicians' large referral pattern. Al-

though this article was not intended to discuss diagnosis, I believe a statement is warranted. The majority of the patients referred to me with Paget's disease have been for "the worst and most stubborn case of monilia vulvitis I have ever seen." The lesion is flat to slightly elevated with a red velvety color within which are intermingled white patches. It very much resembles thrush in babies, which leads to the confusion by the gynecologist. Such gross lesions require early biopsy.

I agree entirely with the authors that frozen sections for margins at operation are indicated and, in fact, approach being mandatory. This lesion always extends beyond its gross margin. Although a 2 cm margin is usually adequate, many times it is not. I also agree that its high recurrence rate is not due to its multifocal nature but rather to inadequate margins. For this reason, I question why the authors recommend simple vulvectomy rather than wide local excision with deep margins with or without a skin graft. The skinectomy procedure has no place in the management of Paget's disease since Paget cells frequently follow hair shafts into the subcutaneous tissues.

I, like the authors, have also never seen a strictly vulvar Paget's disease associated with an underlying adenocarcinoma. This makes the theory of origin of these malignant glandular cells from basal cell metaplasia very attractive. Unlike the authors' experience, I have seen two vulvar Paget lesions invade the underlying stroma. However, both showed only superficial invasion. This would be another excellent reason why frozen sections are so necessary in the treatment of this disease. Both of their recommended techniques for frozen sections are excellent. In fact, I frequently use both techniques in the same patient.

Although the title of this article suggests a limited scope, many hidden messages can be obtained in this report. I encourage this group of private practice oncologists to continue their excellent contributions to our specialty.

DR. BURRELL (Closing). With regard to Dr. May's questions as to what our incidence is, I did go back to try and retrieve the total number of cases of carcinoma in situ of the vulva that we treated in the same time period and I am having a little difficulty coming up with a total figure. It is around 650, so that the 10 patients we had makes a total of 660, which is around 1% to 2%. That figure would be in agreement with the literature. I think our practice does involve referrals from many parts of Georgia, so that we are in fact talking about a fairly large number within the state of Georgia. With regard to the second question, Dr. Underwood, we did indeed follow the theory of wide excision. As it turned out, on six occasions we did a full-thickness vulvectomy because there were bilateral lesions and if one were to take that theory of wide excision one ended up with bilateral vulvectomy. We did not set out with the idea that a bilateral vulvectomy was the standard treatment as opposed to excising all of the lesion, and it turned out that a bilateral vulvectomy was necessary.

Surgical management of distal tubal occlusion

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During the 9-year period ending April, 1985, 103 women had bilateral and unilateral cuff salpingostomy at the University of Virginia Hospital. Currently accepted principles of microsurgery were used as well as an "antiadhesion" regimen consisting of intravenous dexamethasone and antibiotics, intraperitoneal dextran, and postoperative hydropertubations. The postoperative tubal patency rate was 75.6% in tested patients (91.2% in patients known to have conceived). Forty women (38.8%) conceived and 26 (25.2%) have had one or more term pregnancies. Fourteen women (13.5%) had ectopic pregnancies but three of these have also had term pregnancies. Fourteen women (13.5%) had first-trimester abortions. Two of nine women who had repeat salpingostomies have carried pregnancies to term as have two of 10 women who had ampullary salpingostomies. The extent of tubal disease remains the single most important factor with regard to subsequent successful pregnancy. (AM J OBSTET GYNECOL 1986;155:524-30.)

Key words: Salpingostomy, fallopian tube, tuboplasty, distal tubal occlusion

The use of microsurgical techniques¹⁻⁶ for reconstruction of distal tubal occlusion has generally increased postoperative tubal patency rates but has been less effective in improving subsequent live birth rates. The failure of modern surgical methods to match the successes achieved in reversal of sterilization has been attributed to the presence of irreversible postinfectious anatomic and functional abnormalities in terminally occluded oviducts.⁷⁻⁹ Further major improvement in the results of this kind of operation may not be possible. Fortunately, in vitro fertilization and embryo transfer are now more available and expectations of success with this procedure are higher. Some patients may benefit from surgical reconstruction and others may be more appropriate candidates for in vitro fertilization and embryo transfer. Periodic review of accumulated surgical experience may be useful in determining which approach offers the best chance of success in individual cases.

The purposes of this study were to evaluate the surgical techniques used and the perioperative management of our patients, to compare our results with those of previously reported series, and to provide a basis for counseling patients about prognosis for success after distal tubal reconstruction at our institution.

Material and methods

During the 9-year period ending March 31, 1985, 103 women had reconstructive operations by the authors at the University of Virginia Hospital for distal tubal occlusion. All patients had been infertile for at least 1 year (range 1 to 12 years) and all had thorough preoperative infertility evaluations including assessment of ovulatory status, hysterosalpingogram, postcoital test, and evaluation of the male partner by semen analysis and urologic examination. Some patients referred from other physicians had had laparoscopy before referral. Patients from our own practice usually had laparoscopy before proceeding with laparotomy. This diagnostic procedure was performed so that women with false positive hysterosalpingographic findings would not be subjected to unnecessary laparotomy. Patients were not excluded from attempted reconstructive operations because of severe tubal disease and/or extensive adhesions.

The majority of patients in this series have had continued post-operative follow-up by us. Information for this report has been gathered by retrospective analysis of hospital and office records, operative reports, and hysterosalpingograms. Referring physicians have been contacted for recent information about patients lost to follow-up and patients have been contacted directly when possible.

In this study, salpingostomy is defined as the surgical creation of an ostium (cuff) in the distal end of a completely occluded fallopian tube. In a very few cases, a pinpoint opening could be demonstrated at the terminal end of the tube after preliminary lysis of adhesions but all such cases were associated with hydrosalpinx as well as tubal distortion. We have included in this series patients who required a cuff salpingostomy

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on one or both sides. Unilateral salpingostomy was done when the contralateral tube was surgically absent, was involved in bipolar occlusion, or was involved in adhesions to the extent that patency could not be demonstrated before salpingo-ovariolysis.

Before operation was scheduled, all patients and partners wishing to participate were counseled extensively regarding the limited success expected after distal tubal operations. They were advised that operation may not result in patency, that reocclusion might occur, that they would be at increased risk for ectopic pregnancy, that patency might be achieved without subsequent pregnancy, and that extirpative operation may be required or done if judged appropriate.

The surgical procedures were performed according to the generally accepted microsurgical techniques as described by Swolin and Gomel.¹⁻⁴ Dissection was carried out under irrigation with Ringer's lactate and with the use of the microelectrode. The terminal cuffs generally consisted of two or three flaps created by electrosurgical dissection along "avascular lines." In the majority of cases, this part of the procedure was done under loupe magnification and the incisions were extended to a point about 1.5 cm from the distal end of the tube. Serosa-to-serosa sutures of 6-0 to 7-0 polyglycolic acid or polyglactin (four to six sutures) were used in most cases to create the cuff. In a few cases, a single incision (linear terminal cuff salpingostomy) was used. Ampullary salpingostomies (cuff neostomies) were done in essentially the same way in those patients who have previous excision of distal tubes or those in whom distortion and destruction of the distal tubes were such that excision of the most severely affected part was chosen. A pediatric Foley catheter was placed in the endometrial cavity at the beginning of the procedure for chromopertubation during the dissection and for demonstrating tubal patency before closing the abdomen.

An "antiadhesion" protocol was used in more than 90% of patients. This consisted of an antibiotic (usually a penicillin or a cephalosporin) and dexamethasone begun before the laparotomy and continued for 72 to 96 hours. Dexamethasone was given intravenously, 5 mg every 6 hours for 48 hours and 2 mg every 6 hours for an additional 24 hours. The antibiotic was given intravenously every 6 hours for the duration of steroid therapy. Before closing, 150 to 200 ml of dextran (6% dextran 70 or 75) was left in the abdomen. More than 75% of patients had postoperative hydrotubation (with a vacuum hysterosalpingography apparatus) with 25 to 30 ml of the same dextran solution. This was generally done twice during the first 5 postoperative days, 7 to 10 days after operation, and again after the first postoperative menstrual period. Hydrotubation was done in all patients except those who were bleeding, who

Table I. Reconstructive operations for distal tubal occlusion

No. of patients	103
Age (yr) (mean and range)	29.1 (20-38)
Race (No. of patients)	
Caucasian	84
Black	19
Parity (% of patients)	
0	69.7
≥1	30.3
Extent of disease (% of patients)	
Mild	15.7
Moderate	23.5
Severe	37.3
Uncertain	23.5

Table II. Surgical procedures

Operation	No. of patients
Bilateral cuff salpingostomy	49
Ampullary cuff salpingostomy	10
Unilateral cuff salpingostomy	
Solitary tube or contralateral bipolar obstruction	24
With contralateral salpingolysis	13
Repeat salpingostomy	9

Total exceeds 103 since some patients are included in more than one category.

complained of serious discomfort, or who refused the procedure.

The classification of the extent of tubal disease used in this study basically followed that proposed by Rock et al.⁷ with minor modifications. Mild disease included those patients who had recognizable fimbria after patency was achieved, hydrosalpinx of <20 mm in diameter, and minimal peritubal or periovarian adhesions. Moderate disease was defined as hydrosalpinx of 20 to 30 mm in diameter, identifiable but abnormal fimbria after patency was established, and significant adhesions without fixation. Severe disease meant no identifiable fimbria, hydrosalpinx ≥30 mm in diameter, dense adhesions with fixation of ovary and tube, or marked thickening and rigidity of the distal tube. Extent of disease was determined by the status of the less affected tube.

Demographic data concerning the 103 women in this series are depicted in Table I. Mean age was 29.1 years (range 20 to 38). No patients were refused operation because of age. The vast majority of patients were Caucasian and were seen in private practice. The majority of patients had not had a previous viable pregnancy. More than 60% of patients had severe or moderate disease. Extent of disease was uncertain in 23.5% because of incomplete descriptions regarding fimbria in the operative notes but available information suggests that the majority of the cases were severe or moderate.

Duration of follow-up varied from none (did not re-

Table III. Pregnancies after salpingostomy

Group	Pregnant patients		Pregnancy outcome					
			Term		Ectopic		Abortion	
	No.	%	No.	%	No.	%	No.	%
Group 1* (n = 56)	26	46.4	17	30.3	9	16	10	17.8
Group 2† (n = 47)	14	29.7	9	19.1	5	10.6	4	8.5
Total (n = 103)	40	38.8	26	25.2	14	13.5	14	13.5

*Operation before April 1, 1981.

†Operation since April 1, 1981.

Table IV. Duration of follow-up in nonpregnant patients

Group	Duration of follow-up		
	<6 mo	6-24 mo	>24 mo
Group 1* (n = 30)	9	8	13
Group 2† (n = 33)	3	17	13

*Operation before April 1, 1981.

†Operation since April 1, 1981.

turn for post-operative examination) to 12 years. All patients without documented postoperative pregnancy before they were lost to follow-up are counted as treatment failures.

The incidence of associated nontubal, correctable factors that could potentially affect fertility was about 50% and was not significantly different in patients who ultimately conceived and those who did not.

Results

The surgical procedures performed are detailed in Table II. Forty-nine women had bilateral cuff salpingostomy and 37 women had a unilateral cuff as an initial procedure. There were 10 ampullary cuff neostomies and nine women had repeat operations.

Forty of 103 patients treated with bilateral or unilateral cuff salpingostomy are known to have conceived for a total pregnancy rate of 38.8%. Twenty-six women (25.2%) have had one or more term pregnancies. Ectopic pregnancies have occurred in 14 women (13.5% of patients, 35% of patients conceiving). Three of these women have also had term pregnancies but another has had two ectopic pregnancies. Fourteen patients had first-trimester spontaneous abortions (13.5% of patients, 35% of patients conceiving). Four of these have also had term pregnancies.

Since some reports^{2,8,10,11} have suggested that pregnancy may be delayed after distal tubal reconstruction and that cumulative pregnancy rates will improve with long-term follow-up, the study patients were divided into two groups according to the duration of follow-

up. Group 1 included those patients operated on before April 1, 1981 (minimum potential follow-up 4 years) and group 2 included patients operated on April 1, 1981, through March 31, 1985 (maximum possible follow-up 6 months to 4 years). The results are depicted in Table III. The duration of follow-up in patients who are not known to have conceived is shown in Table IV. More than half of those patients in both groups have been followed up <2 years after operation but for greatly different reasons. All such patients in group 1 have been lost to follow-up whereas almost all of those in group 2 are still being followed up.

The mean interval from operation to first conception (expressed in 6-month increments) was 1.8 years (0.5 to 5.5). The mean interval to first term pregnancy was 2.2 years,¹⁴ that to first ectopic pregnancy was 2.1 years (0.5 to 5.5) and that to first abortion was 1.0 years (0.5 to 2.0).

Of those women who conceived, 11 (27.5%) had had a previous term pregnancy. Twenty (31.7%) of those patients who have not conceived had had a previous term pregnancy.

Both the conception rate and the term pregnancy rate are lower in those patients operated on most recently but many patients in the latter group have had very short periods of follow-up.

The mean age of women who conceived after operation was 28.6 years (22 to 34). Patients who had treatment failure had a mean age of 29.5 years (20 to 38) at the time of operation. Patients who failed to conceive after bilateral cuff salpingostomy averaged 28.1 years (20 to 38). A mean age of 30.8 groups (23 to 36) was found in women failing to conceive after unilateral cuffs or repeat operations.

The potential influence of age was examined more closely in the group of patients having bilateral cuff salpingostomy (the largest homogeneous group with respect to surgical procedure). The overall pregnancy rate (33.8%), term pregnancy rate (28.6%), and ectopic pregnancy rate (12.2%) were remarkably similar to those for the entire series. There was no apparent correlation between age at time of operation and subsequent pregnancy (Table V).

Table V. Bilateral cuff salpingostomy—age and outcome

Age (yr)	No. of patients	Pregnant patients		Pregnancy outcome				
				Term		Ectopic		Abortion (No.)
		No.	%	No.	%	No.	%	
≤25	15	6	40	3	20	4	26.7*	0
26-30	21	6	28.6	6	28.6	0		0
31-35	9	7	77.8	5	55.6	2	22.2	1
36-38	4	0		0		0		0
Total	49	19	38.8	14	28.6	6	12.2	1

*One also had a term pregnancy.

Table VI. Unilateral cuff salpingostomy

	Contralateral salpingolysis		Solitary tube or contralateral bipolar obstruction	
	No.	%	No.	%
No. of patients	13		24	
Pregnant patients	6	46.2	8*	33.3
Nonpregnant patients	7	53.8	16†	67.7

*One had combined cuff and isthmic-ampullary anastomosis.

†Two had ampullary salpingostomy in a solitary tube.

Thirteen women had a unilateral cuff salpingostomy and a contralateral salpingolysis. Six (46.2%) of these women have conceived. Another 24 had unilateral cuff salpingostomy in a sole remaining tube or in the presence of contralateral bipolar obstruction. Eight (33.3%) of these women have conceived. This group of patients includes one who had a combined cuff salpingostomy and isthmic-ampullary anastomosis in a sole remaining tube (and who subsequently had an ectopic gestation) and two who had an ampullary salpingostomy in a solitary tube (Table VI).

The results of repeat operations for distal tubal occlusion are shown in Table VII. Nine patients had repeat bilateral cuff salpingostomy or a unilateral cuff in a solitary tube. Two patients (22.2%) have conceived and carried pregnancies to term although one has also had an ectopic pregnancy.

Cuff neostomies (ampullary salpingostomies) were performed in 10 women who had had previous excision of distal tubes for sterilization or whose distal tubes were excised because of severe thickening and rigidity. Nine of these procedures were bilateral and one was unilateral and combined with a conventional cuff salpingostomy on the opposite tube. Four (40%) of these patients have conceived and two have had term pregnancies. Only one of the six women operated on after previous sterilization has conceived but that pregnancy was carried to term.

Table VII. Results of repeat salpingostomy

	Pregnant		Not pregnant
	Term	Ectopic	
No. of patients (n = 9)	2	1*	7

*Also had a term pregnancy.

Postoperative tubal patency as assessed by hysterosalpingography (usually done 2 to 4 months after operation) was examined (Table VIII). Ninety patients had this procedure done and 68 had demonstrated patency of one or both tubes (75.6%). However, in those patients who have subsequently conceived, the patency rate was 91.2% as compared with 66.1% in those not yet pregnant.

The influence of the extent of tubal damage (least affected tube) on subsequent pregnancy rates was examined (Table IX). The significance of this information is obscured by the relatively large number of cases in the uncertain category. However, if it is assumed that the distribution of the extent of damage is the same in this group as in the remaining patients, it appears that severe tubal damage (even as assessed by this relatively arbitrary method) is associated with the lowest subsequent conception rate.

Postoperative morbidity was rare in this series. Six women had a mild and transient fever that resolved without evidence of intraperitoneal infection. All patients were discharged by the sixth postoperative day. There was no apparent morbidity associated with the "antiadhesion" adjuvants.

Comment

There are numerous difficulties in attempting to compare the results of reported series of distal tubal operations. Criteria for patient selection are not uniform. Various surgical techniques have been used and success rates may be defined differently. A classification of operations has been published³ but has not been strictly adhered to in recent reports.

Table VIII. Postoperative tubal patency by hysterosalpingography

	Pregnant patients (n = 40)	Nonpregnant patients (n = 63)
Bilateral patency	19	14
Unilateral patency	9	16
Solitary tube patent	3	7
Solitary tube occluded	1	5
Bilateral occlusion	2	14
Not done	6	7
One or both tubes patent in tested patients	31 (91.2%)	37 (66.1%)

Table IX. Pregnancy and extent of disease

Extent of disease	No. of patients	Pregnant patients						Nonpregnant patients	
		Total		Term		Ectopic			
		No.	%	No.	%	No.	%	No.	%
Mild	16	3	50	4	25	4	25	8	50
Moderate	24	11	45.8	8	33.3	5	20.8	13	54.2
Severe	38	9	23.7	7	18.4	—	—	29	76.3
Uncertain	25	12	48	7	28	5	20	13	52

We have chosen to use the term salpingostomy to describe the creation of a cuff in the distal end of a completely or nearly completely occluded fallopian tube. This definition implies that full-thickness incisions have been made through the tubal wall and that serosa-to-serosa sutures have been used to create a cuff. We have not included patients who had other surgical procedures involving the distal tube unless they were unilateral and combined with a contralateral salpingostomy.

Our overall success rate compares favorably with recently reported series in which microsurgical techniques have been used^{1, 2, 8, 9, 12-14} with regard to both overall conception rate (38.8%) and live birth rate (25.2%). It may be argued that these figures are inflated by inclusion of some patients who had unilateral salpingostomy combined with contralateral salpingolysis. However, as is apparent from Table V, the conception rate was the same and the live birth rate even higher (28.6%) in the subset of patients who had bilateral cuff salpingostomy. The patients with the poorest outlook are those who had unilateral cuff salpingostomy in a solitary tube or if there was bipolar occlusion of the contralateral tube (Table VI). The ectopic pregnancy rate (13.5%) is also comparable to that of other series and obviously represents a major potential complication of this kind of surgery. Some authors^{2, 8} have pointed out that the increase in conception rates that has presumably resulted from microsurgical techniques has been accompanied by an alarming increase in ectopic pregnancy rates. This result would not be unexpected if current surgical principles do result in a

high rate of tubal patency without concomitant normal anatomy and function.

The most important prognostic factor according to most authors is the condition of the tube. The classification system that we have used to describe extent of disease in this series is consistent with this observation. However, since any such classification is to some extent arbitrary and since adequate information for accurate classification is not usually available until the operation has been done, this information is more useful for prognosis after operation than for deciding whether or not to operate. Many other variables including operative technique, patient selection, and experience of the surgeon may affect success rates.

Most authors have observed that conception after distal tubal operations is frequently delayed and that cumulative pregnancy rates will increase with continued follow-up. Our data tend to support this observation. It may be that time is required for restoration of tubal anatomy and function.⁴ Whatever the reason, it is apparent that expectations for delayed success will be more acceptable to younger patients and that older couples may elect in vitro fertilization and embryo transfer after a shorter postoperative interval.

It is not known whether pharmacologic adjuvants or hydroperturbation enhances microsurgical technique. We have used both in the majority of patients in this study. One recent controlled study did not show an improved term pregnancy rate for terminal salpingostomy when perioperative dexamethasone was used. We did not recognize any morbidity associated with the use of dexamethasone in this series. A sense of well-being

among these patients, presumably attributable to the corticosteroid, was uniformly observed. Similarly, we did not recognize any morbidity associated with hydropertubation. In a small series, Spadoni¹³ showed a marked increase in pregnancy rate when this procedure was used.

Second-look laparoscopy was not done routinely in this series. The potential benefits of this procedure include documentation of reocclusion and opportunity for laparoscopic lysis of adhesions and/or repeat salpingostomy.^{1, 2, 9, 15} A recent large series¹⁵ in which laparoscopy was done on the eighth postoperative day after distal tubal operation revealed a high incidence of adnexal adhesions, more than half of which were successfully lysed. It was concluded that the occurrence of permanent pelvic adhesions was significantly diminished and that the incidence of subsequent ectopic pregnancy was significantly lowered by this procedure.

The results of repeated tuboplasties have been reviewed recently.¹⁶ The prognosis is poor in such cases but among the nine cases in the present series two women have had term pregnancies. Candidates for repeat salpingostomy as well as those who have had previous distal excision (for instance, for sterilization) may still be acceptable surgical candidates if other options are not available.

The laser was not used in any patient in this series. It is not yet established whether the rate of subsequent live births will be improved by use of the laser. One recent report suggests that there is no difference in the pregnancy rate and in the operation-conception intervals between salpingostomy with the carbon dioxide laser or that with electrosurgery.

The success rate for in vitro fertilization and embryo transfer in some centers is now approaching that expected for live births after distal tubal reconstruction. Furthermore, pregnancies after in vitro fertilization and embryo transfer occur sooner, not years later, and a minor rather than a major operation is required. To date, ectopic pregnancy has been uncommon in in vitro fertilization and embryo transfer programs. There is now more than one reasonable option available for patients with distal tubal occlusion. After appropriate counseling, they should participate in the decision as to whether to attempt in vitro fertilization and embryo transfer or have reconstructive operations. Some of the factors that may influence this decision include age, financial considerations (including insurance coverage), the importance of enhancing the probability of an immediate result, and willingness to assume the significant risk of a potentially catastrophic outcome after operation (for instance, ectopic pregnancy). Since successful operation carries with it the possibility of subsequent repeated spontaneous pregnancies, it is likely that younger patients will continue to request this ap-

proach, at least initially. For those couples, in vitro fertilization and embryo transfer remains an option for later. Older couples may prefer in vitro fertilization and embryo transfer sooner but may also be candidates for operation later if pregnancy is not achieved.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. L. LESLIE WASSERMAN, JR., Virginia Beach, Virginia (Official Guest). The clinical dilemma of trying to restore normal reproductive function to fallopian tubes ravaged by pelvic inflammatory disease is among the most frustrating faced by the gynecologic surgeon. Successful conception requires not only a patent fallopian tube but also a fully functional one. This assumes normal fimbriae, normally functioning cilia throughout the endosalpinx, and a thin, flexible myosalpinx. Pelvic inflammatory disease disrupts the tubular integrity at every level. Pregnancy rates after salpingoplasty are significantly higher in post-tubal ligation patients than in those with pelvic inflammatory disease. In addition, microsurgical techniques have improved outcome mainly in the post-tubal ligation group.

Success in tuboplasty has always been based on strict surgical principles including meticulous attention to perfect hemostasis, the use of fine sutures with exceedingly low tissue reactivity, gentle handling of tissues, and vigorous attempts to minimize postoperative adhesion formation. The movement toward microsurgical approaches would seem to improve the desired result, but a review of the literature during the past 5 years shows little consistent increase in efficacy in restoring chronically inflamed tubes.

The situation of distal tubular occlusion has a particularly poor prognosis. Those articles investigating this entity over the past several years have shown post-surgical term pregnancy rates of between 5.6% and 41%. Again no significant difference has been obtained when microsurgical techniques have been used. Various adjuncts have been tried including preoperative, intra-operative, and postoperative antibiotics and/or steroids, intraperitoneal dextran, and postoperative hydrotubation. There continues to be disagreement in the literature as to the effectiveness of these regimens as well as to which medications should be used in each of them.

Dr. Kitchin and his associates have operated on 103 patients with distal tubular occlusion during a 9-year period. In this study were included bilateral and unilateral cuff salpingostomies performed under loupe magnification. Patients with other infertility causes were carefully excluded. A majority of the patients received preoperative and postoperative antibiotics and steroids as well as intraoperative 6% dextran 70 or 75 and postoperative hydrotubation. There was an overall total pregnancy rate of 38.8%, a term pregnancy rate of 25.2%, and a 13.5% ectopic pregnancy rate. The figures improved in those patients followed up for >4 years to a total pregnancy rate of 46.7%. Another subgroup showing an improved success rate was that segment of patients requiring unilateral cuff salpingostomy with contralateral salpingolysis. A 46.2% total pregnancy rate was found in the 13 patients composing this group.

The data presented here verify the poor success rate expected with attempted surgical correction of distal tubular occlusion. This article presented no new answers as to whether antibiotics, steroids, dextran, postoperative hydrotubation, or, indeed, the microscopic approach offers any real improvement in term pregnancy rate. Dr. Kitchin's concern as to the patient's choice between cuff salpingostomy and in vitro fertilization is well placed in my opinion. For the couple wanting quicker results, a lower risk of ectopic pregnancy, and avoidance of a major operation with its financial and risk ramifications, in vitro fertilization may well be the better choice. Dr. Kitchin's study is appreciated as his experience joins the growing body of evidence verifying the difficulty in making real progress in the area of surgical correction of distal tubular occlusion. My two questions for Dr. Kitchin would concern first the legitimacy of including unilateral cuff salpingostomy with contralateral salpingolysis in this study as it is clearly a subgroup with a more favorable

prognosis and second whether some of the procedures performed in which there was a pinpoint opening in the terminal end of the tube might not better have been classified as fimbrioplasties following the recommendations of the International Federation of Fertility and Sterility published in 1980.

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DR. WILLIAM L. AMOS, Columbus, Georgia. I would like to ask Dr. Kitchin, in view of his offering in vitro fertilization as an alternative, if he can give a rough idea of what percentage of these patients' ovaries would have been accessible for laparoscopic ovum retrieval without laparotomy and lysis of adhesions.

DR. DOROTHY MITCHELL, Atlanta, Georgia. I would like to ask Dr. Kitchin two questions. One is whether he noted a difference in the mucosal pattern at the time of laparotomy and procedure as far as the outcome. Second, in light of the new procedure called Gift, that is, fallopian transfer with a need for patent tubes, which may give an additional method of in vitro fertilization, perhaps this may be more an adjunctive procedure and may not incur the cost of an in vitro fertilization.

DR. WILLETT L. LEHEW, Norfolk, Virginia. There have been some recent studies of the liberal use of early postoperative laparoscopy to lyse adhesions. I wonder if Dr. Kitchin has had any experience with this.

DR. KITCHIN (Closing). Dr. Amos, were you asking whether early laparoscopy is desirable for purposes of lysing adhesions? I missed your question. That is addressed in the article. We have not routinely done second-look laparoscopy. Some people have recommended it for some time and I think there is an increasing body of evidence to support it, in fact, within the past 6 months, there is a relatively large series reported, I believe, in *Fertility and Sterility*, from Germany, in which several patients had postoperative laparoscopy on the eighth day and about half of those patients were found to have significant adhesions. Lysis was performed and it was the opinion of the authors that the outcome, with regard to both subsequent live births and a diminished incidence of ectopic pregnancy, was improved. Of course, there are many things you can say about that. We do not know what happens to adhesions that are there on the eighth day, whether they are still there a month or 6 months or 9 months later, but if you get rid of them, hopefully they will be gone. Dr. Mitchell asked questions about the mucosal pattern and whether observations were made presumably with regard to the state of the rugae at the time of operation. We did make an attempt to classify the patients at the time of operation with regard to the presence or absence of rugae and it did not change the incidence of mild, moderate, or severe adhesions. I think that this

is an observation that certainly can and should be made at the time of operation. With regard to the possibility of using the Gift procedure, in many of these patients I certainly share enthusiasm for that and I think that many of these patients may, in fact, be candidates for Gift rather than in vitro fertilization-embryo transfer. With regard to the question of whether the ovary was accessible to ovum retrieval in the patients, at the time that we closed the abdomen, I would say that virtually all of these patients had one or both ovaries accessible for laparoscopic retrieval of ova. However, as you implied in your question, I think if the operation were not done it may not be that such availability would exist for translaparoscopic ovum retrieval. So I think that if you are making the point that a surgical procedure may be required whether or not it is anticipated that you are going to wait for spontaneous pregnancy after operation, I would agree with that. In fact, we would recommend that if you do this procedure you make every effort to leave the ovary accessible in the event that in vitro fertilization-embryo transfer is required later. Dr. Wasserman certainly deserves my thanks for reviewing this article. I appreciate very much his comments. He asked whether it would be legitimate to include the patients with a unilateral cuff and that certainly is a good point. I think that the way we have tried to get around that criticism would be to show you our

results with a unilateral cuff procedure separate from the results of the other article as a whole and I would accept that as a legitimate criticism. With regard to the operation, and I am glad he asked it, as to whether we should have differentiated between so-called fimbrioplasty and salpingostomy, I am delighted to address that. I really cannot agree with that classification. First of all it is a classification that can only be made after you have done the operation, which puts you in the unusual position of not knowing what you have until you have done it. But, of course, all of us who have been in the operating room are familiar with that feeling. Also, it gives a name to a procedure, depending on what you have accomplished in the operating room. It would be, in my opinion, sort of like calling a hysterectomy a hysterectomy if you did it for carcinoma of the endometrium but calling it something else if you did it for endometriosis or in the course of doing a repair. So, to me, and I tried to address this a couple of times in our presentation, salpingostomy is what I define it as. It is opening the end of an occluded tube and if you want to call it a fimbrioplasty based on the way the fimbria looks after you get the tube open, then that is okay, but I think the classification of the extent of disease that we have tried to use takes that into consideration. I think this is a question of semantics but I understand the criticism.

Adult-onset amenorrhea: A study of 262 patients

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A series of 262 patients with amenorrhea of adult onset are reported. Hypothalamic suppression followed by inappropriate positive feedback, and then hyperprolactinemia and ovarian failure are the most frequently encountered etiologies. Other etiologies are diverse and numerically less frequent. Amenorrhea after use of oral contraceptives, or postpill amenorrhea, occurred in 77 (29%) of all patients. The average age of presentation, prior menstrual history, associated morbidity, and subsequent reproductive potential of each diagnostic group are reported. Adult-onset amenorrhea has a less significant impact on future well-being than was reported for a similar-sized group of patients whose amenorrhea developed as a result of pubertal aberrancy. (AM J OBSTET GYNECOL 1986;155:531-43.)

Key words: Secondary amenorrhea, postpill amenorrhea

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The absence of menstrual function during reproductive years is a relatively infrequent occurrence. For the Swedish female population of ages 18 to 45 years, an epidemiologic study calculated the 1-year incidence rate of secondary amenorrhea of at least 6 months' duration to be 0.7%.¹ Amenorrhea represents a bio-

logic marker that warns of potential health hazards. The presence or absence of menses before the onset of amenorrhea provides little additional information about the significance of this menstrual phenomenon, since the same etiologies may be present with either primary or secondary amenorrhea.² Many of the causes of amenorrhea are either related to neuroendocrine maturation at puberty or become evident during the normal pubertal years. Other problems develop independent of neuroendocrine maturation or at a time more removed from the pubertal process.

A series of 252 patients evaluated at the Medical College of Georgia from 1960 to 1980 for pubertal aberrancy has been reported.² Criteria for inclusion into that retrospective series consisted of delay in the onset of pubertal development (that is, absence of thelarche by age 13), abnormalities in the sequence and tempo of pubertal development (for example, menarchal delay by 15 years), and secondary amenorrhea following an arbitrary six cycles or less of menses after menarche. This last criteria was added to include those chronic anovulation patients who did not develop an appropriate positive feedback mechanism during puberty. Patients with any form of pubertal aberrancy could also be labeled with pubertal amenorrhea: the absence of menses that becomes clinically manifested in and around pubertal maturation. The present study reports on 262 patients evaluated at the same institution during the same time period for amenorrhea of adult onset. The purpose of this study is to contrast the two groups of patients: pubertal amenorrhea versus adult-onset amenorrhea. The etiologic factors of 262 patients presenting to a tertiary center for adult-onset amenorrhea, the nature of their presentation, and associated abnormalities are examined in this paper. This present series of patients with adult-onset amenorrhea may present the last opportunity to study a large number of such patients in as much as evaluation of these individuals today is often initiated before 6 months of amenorrhea.

Material and methods

From 1960 to 1980, 262 patients were evaluated in the Reproductive Endocrine Unit of the Medical College of Georgia because of adult-onset amenorrhea. Patients were referred from gynecologists in Georgia and surrounding areas. Criteria for inclusion into this retrospective study consisted of secondary amenorrhea of 6 months' duration or more which was preceded by at least six menses after menarche and which occurred before age 39 years.

The availability of gonadotropin and prolactin determinations by radioimmunoassay, the development of banding techniques for cytogenetic studies, and advances in other diagnostic procedures occurred during this 20-year span. The diagnostic approach to each of

these patients depended on the time period during which evaluation occurred.

The initial evaluation included assessment of prior pubertal development, menstrual and medical histories, ascertainment of genital and somatic anomalies, and determination of estrogen status by vaginal cytology and/or a progesterone challenge test. The progesterone challenge test was considered an adequate reflection of good endogenous estrogen if it resulted in withdrawal bleeding which approximated prior spontaneous menses. Short episodes of spotting following progestin administration were considered to reflect a relative hypoestrogenic state. Pregnancy tests were performed when appropriate to rule out the presence of trophoblast. Pregnant patients were excluded from this study.

Gonadotropin assays were performed on all patients who were hypoestrogenic. In recent years dynamic pituitary function studies with 100 µg of gonadotropin-releasing factor, insulin (1.0 to 1.5 U regular per kilogram of body weight), and 250 µg of thyrotropin-releasing factor have been used for patients in whom pituitary dysfunction or subclinical hypothyroidism was considered. More recently, 25 U of corticotropin has been given intravenously to identify the patient with adult-onset congenital adrenal hyperplasia.

Cytogenetic studies of peripheral blood were performed on all individuals with elevated gonadotropin assays. Earlier studies used the standard method of Moorehead and associates. G-banding and C-banding were performed in recent years. A minimum of 20 cells were counted in each individual, and a minimum of seven cells were karyotyped. In patients in whom mosaicism was suspected, at least 50 cells were evaluated. Buccal smears were evaluated in these same patients.

Lateral and posteroanterior skull films were obtained in all patients who were hypoestrogenic. Prolactin assays have been added in recent years for the evaluation of patients suspected of having pituitary tumors. Abnormalities of either of these tests or the presence of symptoms suggesting tumor prompted further radiographic evaluation. Such studies have varied over the years and have involved hypocyloidal polytomography, arteriography, pneumoencephalography, and third-generation computerized tomographic scanning. Since multiple prolactin values were obtained in patients with hyperprolactinemia, only the initial values are reported here. Subsequent values were essentially concordant for all of these patients.

Thyroid studies were performed in all patients with hyperprolactinemia and in any patient suspected of having primary thyroid disease. The patients suspected of having Cushing's syndrome, congenital adrenal hyperplasia and recycling failure with androgen overproduction underwent appropriate hormone assays.

Table I. Classification of patients with adult-onset amenorrhea

Hypogonadism (hypoestrogenic)
Hypergonadotropic hypogonadism
Chromosomally incompetent ovarian failure
Chromosomally competent ovarian failure
Hypogonadotropic-eugonadotropic hypogonadism
Reversible
Irreversible
Eugonadism (estrogenic)
Anatomic genital abnormalities
Inappropriate positive feedback

All patients were evaluated for possible associated anomalies. Intravenous pyelography, cardiac evaluation, and thyroid studies were performed in all patients with abnormal karyotypes.

Results

The patients were grouped according to estrogen status (gonadal function) with the use of a modification of the classification system described for pubertal amenorrhea (Table I).² Hypogonadal patients (hypoestrogenic) were further subdivided according to gonadotropin status: hypergonadotropic hypogonadism and hypogonadotropic-eugonadotropic hypogonadism comprise these categories.

Patients with ovarian failure (hypergonadotropic hypogonadism) were considered to have chromosomally incompetent ovarian failure if cytogenetic studies revealed any privation of X chromosomal material. Phenotypic females with 46,XX karyotypes associated with ovarian failure were considered to have chromosomally competent ovarian failure. Hypogonadal patients with gonadotropin values that were low or within the normal menstrual range (hypogonadotropic-eugonadotropic hypogonadism) were considered to be hypoestrogenic secondary to interference with the pulsatile release of gonadotropin-releasing hormone or with gonadotropin secretion. Reversible causes of hypogonadotropic-eugonadotropic hypogonadism consisted of anorexia nervosa and other forms of weight loss, hypothalamic etiologies (for example, exercise, stress), hypoestrogenic forms of endocrine disorders (that is, hypothyroidism, Cushing's disease, hyperandrogenic states), and hyperprolactinemia. Irreversible causes included forms of panhypopituitarism (for example, Sheehan's syndrome) and acquired anatomic lesions (pituitary tumors, postsurgical hypopituitarism, and empty-sella syndrome). Patients with estrogenic forms of amenorrhea were divided into two eugonadal groups: anatomic and inappropriate positive feedback. The latter group included patients with chronic anovulation of the polycystic ovarian disease type as well as other women with problems which adversely affected ovu-

Table II. Etiologic breakdown of 262 patients presenting with adult-onset amenorrhea

	Group total	n	%
Hypergonadotropic hypogonadism			
Chromosomally incompetent ovarian failure	4	4	.5
Chromosomally competent ovarian failure (46,XX)	27	27	10.0
Total	31		12.0
Hypogonadotropic-eugonadotropic hypogonadism			
Reversible (potentially)	104		*
Hypothalamic: weight loss/anorexia		41	15.5
Hypothalamic: nonspecific		27	10.0
Hypothyroidism		2	1.0
Hypothyroidism/hyperprolactinemia		1	0.5
Cushing's syndrome		2	1.0
Hyperandrogenic states			
Undiagnosed		1	0.5
Ovarian tumor (adrenal rest)		2	1.0
Adult-onset congenital adrenal hyperplasia		1	0.5
Hyperprolactinemia			
Tumor and/or prolactin greater than 100 ng/ml		20	7.5
Prolactin >30 and <100 ng/ml		3	1.0
Drug-induced		4	1.5
Irreversible	7		
Tumor (unclassified)		1	0.5
Empty sella		2	1.0
Sheehan's syndrome		4	1.5
Total	111		42.0
Eugonadism			
Anatomic (Asherman's syndrome)	18	18	7.0
Inappropriate positive feedback	102		
Chronic anovulation		73	28.0
Hypothalamic		20	8.0
Hyperprolactinemia		8	3.0
Empty sella syndrome		1	0.5
Total	120		46.0

lation but did not prevent estrogen production (for example, mild hypothalamic suppression or hyperprolactinemia).

Hyperprolactinemic patients were divided into five categories. Category 1 patients had prolactin values >100 ng/ml and/or radiographic evidence of a pituitary tumor. Category 2 patients had prolactin values between 30 and 100 ng/ml and did not have evidence of tumor. Category 3 patients had hyperprolactinemia concomitant with an estrogenic form of amenorrhea. Category 4 patients had drug-induced hyperprolactinemia, and category 5 patients had empty-sella syndrome diagnosed by pneumoencephalogram or computerized tomographic scan.

Patients were labeled with postpill amenorrhea if their absent menses of at least 6 months' duration was immediately preceded by the usage of oral contraceptives for a minimum of 6 months.

Table III. Clinical data in all categories

Category	No. of patients	Average age of presentation (yr)	Estrogen status*	Menarche (yr)		Prior regular menstrual cycles (% of patients)	Prior pregnancies (% of patients)	Pregnancy loss	
				Range	Mean			n	%
Chromosomally competent ovarian failure	4	29.25	—	13, 13, 12.5, 12	12.6	75	50	5/6	83
Chromosomally competent ovarian failure (46,XX)	27	25.1	—	11-16.5	12.8	70	15	2/10	20
Weight loss anorexia	41	21.9	—	10-15	12.5	89	12	3/7	43
Nonspecific hypothalamic (I)	27	24.7	—	10-15	13.2	80	41	6/17	35
Nonspecific hypothalamic (II)	20	21.8	+	12-16	13.5	59	26	7/10	70
Hypothyroidism	2	18, 25	—	17, 13		100	50	0/1	0
	1	34	—	15		100	—	—	—
Cushing's syndrome	2	19, 20	—	12.5, 12		100	—	—	—
Hyperandrogenic Undiagnosed	1	30	—	12		—	—	—	—
Ovarian tumors	2	18, 25	—	13, 13		50	50	2/2	100
Congenital adrenal hyperplasia	1	19	—	11		100	—	—	—
Category of hyperprolactinemia I	20	25.5	—	10-16	13	65	25	1/9	11
II	3	30	—	17, 13, 13	14.3	66	30	0/2	0
III	8	23.14	+	11-14	12.3	63	50	1/6	16
IV	4	26	—	12-16	13.7	75	25	0/2	0
V	2	25, 21	—	12, 13		50	50	0/1	0
V	1	38	+	12		100	‡	0/6	0
Tumor, unclassified	1	19	—	12		100	—	—	—
Sheehan's syndrome	4	31.75	—	11, 12, 12, 13	12	100	100	4/21	19
Asherman's syndrome	18	23.8	+	11-13	12.4	100	100	15/42	36
Inappropriate feedback	73	22	+	9-16	12.3	55	24	10/31	32
Total	262	25.4	§		12.87	71	31	56/173	32

ND, No data; NA, not available.

*+ = Positive. — = Negative.

†Number in parentheses indicates number of cases.

‡Six pregnancies.

§Negative: 142 cases (54%).

Oral contra- ceptive amen- orrhea		Duration of amenorrhea at presentation (mo)		Influencing factors†	Prolactin (ng/ml)		Morbidity‡	Reproductive potential
n	%	Range	Mean		Range	Mean		
—		6-132	39				Thyroid carci- noma (1)	Significantly reduced
10	37	6-144	46.4				Associated thyroid carcinoma (1)	Significantly reduced
18	43	6-48	16.8	Weight loss of 2 to 100 pounds; average, 29.9 pounds			Diabetes (1)	With counseling and ovulation in- duction
13	52	6-108	30	Emotional disorders (10), sys- temic disorders (4); exercise (2)			Anorexia nervosa (4)	With counseling and ovulation in- duction
6	30	6-48	17.2	Weight loss, 10-30 pounds, average, 17.5 (14); and ex- ercise related (1); emotional disorders (3); systemic dis- orders (2)			0	With counseling and ovulation in- duction
—		12, 13			19, 124		0	Usually with thyroid replacement
—		24		Associated hyperprolactinemia unresponsive to thyroid treatment	75		0	
—		6, 24		None	33		ND	With adrenalectomy and replacement
—		84		Diabetes mellitus			Diabetes mellitus	With ovulation in- duction
—		36, 24		Both adrenal rest tumors			Ovarian tumors (2)	With surgical re- moval
—		9						With adrenal suppression and/ or ovulation in- duction
6	30	6-144	37.9	Estradiol pellets (2) (prolactin, 1500, and 1600 ng/ml)	68-1600	385.12	Tumors (12)	With prolactin suppression and/ or ovulation in- duction
2	66	15, 26, 13	18		96, 70, 87	84.3		With prolactin suppression and/ or ovulation in- duction
4	50	6-72	38.14		51-130	77.2		Same
—		6-24	12		40-94	67.3		
1	50	14, 24		Pseudotumor cerebri (1)	42, 200			With ovulation in- duction
—		8		Galactorrhea	NA			With ovulation in- duction
—		120		Hypophysectomy (1966) chromophobe adenoma	NA		Tumor	With ovulation in- duction (Pergonal)
—		12, 18, 60, 216	76.5	Traumatic vaginal deliveries (2) postpartum hem- orrhage			Panhypopitui- tarism (4)	With ovulation in- duction (Pergonal)
2	10.5	6-60	21	Curettage after postpill amen- orrhea (1 case)				
15	21	6-156	21.6					With ovulation in- duction
77	29.2		22.12					12% potential abso- lute barrier

Once classified, patients were further evaluated for prior menstrual function and reproductive function, morbidity and mortality secondary to the disease state, and maximum future reproductive function. Patients were considered to have regular cycles if menstrual intervals ranged between 21 and 40 days.

Table II lists the number of patients in each category and the breakdown of these groups by percentages of total patients evaluated. Table III lists the manner of presentation and the findings associated with each of these categories evaluated. Only significant findings are discussed in the text.

Hypogonadotropic hypogonadism. Thirty-one patients were found to have ovarian failure. Four (13%) of these individuals had chromosomally incompetent ovarian failure, and 27 (87%) had chromosomally competent ovarian failure (Table II).

Chromosomally incompetent ovarian failure. Karyotypes of the four patients with X chromosome privations are: 46,X,i(Xq) (two patients); 45,X (52%)/47,XXX (48%); 45,X (94%)/46,XX (6%). Table III summarizes the manner of presentation of these chromosomally incompetent patients. Three of these patients had prior regular cycles. The two patients with mosaicism had achieved pregnancies on three occasions each. One patient presented with recurrent abortion and amenorrhea and was found to have a 45,X/46,XX karyotype. She had spontaneous cycles through her thirty-sixth birthday. The other patient had one early spontaneous abortion, the delivery of a normal 46,XX infant, and then at age 26 years another spontaneous abortion. The height for these patients was 53 3/4 inches, 57 inches, 60 inches, and 60 inches (mean, 57.63 inches). Turner stigmas were variably present. None of these women had cardiovascular anomalies. One patient with the 46,X,i(Xq) karyotype had previously been treated for a thyroid carcinoma metastatic to paracervical lymph nodes.

Chromosomally competent ovarian failure. A well-defined etiology was unable to be elicited in any of these patients. All patients were found to have a 46,XX karyotype. Manner of presentation of the chromosomally competent patients is summarized in Table III; 30% of them had prior regular cycles and 15% had previously achieved a pregnancy. Spontaneous resolution of the ovarian failure was subsequently reported in three (11%) of the patients with chromosomally competent ovarian failure. One individual was delivered of a male infant 2 years after diagnosis of ovarian failure. Height for these patients ranged from 60 to 68 inches with a mean of 66.32 inches. Hypothyroidism was diagnosed in one patient. One patient had previously had the resection of a neck tumor (epithelioma), and a second individual was found to have a thyroid carcinoma. Other concomitant systemic illnesses reported included

juvenile-onset diabetes mellitus in one patient and tuberculosis in another.

Hypogonadotropic/eugonadotropic hypogonadism. One hundred eleven patients were found to be hypogonadotropic secondary to a hypothalamic-pituitary problem. One hundred four (94%) of these individuals had reversible causes, and seven (6%) had irreversible conditions (Tables II and III).

Reversible conditions. Sixty-eight patients had some form of hypothalamic suppression. Forty-one of these patients reported an associated weight loss averaging 29.9 pounds. Anorexia nervosa was diagnosed in four patients who lost separately 25, 50, 57 and 100 pounds. Nonspecific hypothalamic suppression occurred in 27 of these individuals. Ten patients had known stress or emotional disorders. Two patients were joggers. Systemic illnesses were reported in four patients (thyroid nodule, renal failure, hypertension, and juvenile-onset diabetes mellitus). Of these patients with hypothalamic suppression 80% to 90% had prior regular cycles (Table III). Pregnancy had been achieved in 12% of the patients with weight loss and in 4% of individuals with nonspecific forms of hypothalamic suppression. Pregnancy loss occurred in 43% of the gestations in the weight loss group and 35% of the nonspecific hypothalamic suppression gestations.

Other reversible forms of hypogonadotropic-eugonadotropic hypogonadism included hypothyroidism (two patients), combination hypothyroidism/hyperprolactinemia (one patient), Cushing's syndrome (two patients), and hyperandrogenic states (four patients). The patient with combination hypothyroidism/hyperprolactinemia had thyroxine values below normal limits which responded to thyroid replacement; however, prolactin levels remained elevated. Hyperandrogenic hypogonadotropic states were noted in two patients with adrenal rest tumors, in one patient with adult-onset congenital adrenal hyperplasia, and in another woman who presented with chronic anovulation and diabetes mellitus. Table III lists presenting historic information for these individuals. Hyperprolactinemia was considered to represent a potentially reversible condition and was reported in 27 patients. Twenty patients were subgrouped into category 1. Prolactin values ranged from 68 to 1600 ng/ml with a mean of 385.12 ng/ml for these patients. Twelve of them had identifiable tumors. Sixty-five percent of these patients had prior regular cycles, and 25% had achieved pregnancies. Three hypogonadotropic patients were placed in category 2 because of prolactin values of 96, 70, and 87 ng/ml. Two of these patients had prior regular cycles, and one had achieved two term pregnancies. Drug-induced hyperprolactinemia (category 4) was reported in four patients whose prolactin levels ranged from 40 to 94 ng/ml and averaged 67.3 ng/ml, three of whom had prior regular

cycles and one had two term pregnancies. The drugs taken by these four patients were Prolixin, Artane and Triavil, phenobarbital and another unknown tranquilizer, and Thorazine, respectively.

Irreversible conditions. One patient had a chromophobe adenoma resected in 1966 and presented postoperatively with over 10 years of amenorrhea. Prolactin assays were unavailable at the time of tumor diagnosis.

Empty-sella syndrome associated with hyperprolactinemia (category 5) was diagnosed in two hypogonadal patients. One of these patients had prior regular cycles and one term pregnancy, and she later developed pseudotumor cerebri. Presenting prolactin value was 200 ng/ml. The other patient never had regular cycles and presented with a prolactin level of 42 ng/ml.

Sheehan's syndrome was diagnosed in four women (Table III) who had a total of 21 pregnancies with a 19% loss rate. The last pregnancy ended in a traumatic delivery and significant blood loss for two of these women and postpartum hemorrhages for the other two patients. Typical signs of panhypopituitarism followed for all four patients, and endocrine data supported the diagnosis of Sheehan's syndrome.

Eugonadism. One hundred twenty patients were found to have amenorrhea associated with gonadal steroid production. Anatomic defects were found in 18 (15%) of these patients, and inappropriate positive feedback was diagnosed in 107 (85%) (Tables II and III).

Asherman's syndrome. All of these patients had regular cycles and prior pregnancies. Thirty-six percent of prior pregnancies resulted in early gestational loss (one premature, 14 abortions). Two patients had been placed on oral contraceptives post partum and subsequently stopped having withdrawal menses. Amenorrhea persisted in both. However, one of these women had a dilatation and curettage while taking the oral contraceptives for diagnosis of the amenorrhea.

Inappropriate positive feedback. Identifiable causes of anovulation were noted in 29 patients: estrogenic forms of hypothalamic suppression (20); hyperprolactinemia (eight); and empty-sella syndrome (one). The eugonadal group of patients with hypothalamic suppression consisted of 14 women with an average weight loss of 17.5 pounds, one individual who jogged, three patients with emotional distress, and two women with systemic disorders (that is, seizures and pseudotumor cerebri). Twenty-six percent of women with estrogenic hypothalamic suppression had previously become pregnant. However, seven of 10 of these pregnancies were lost. The estrogenic hyperprolactinemic patients (category 3) had prolactin values ranging from 51 to 130 ng/ml and averaging 77.2 ng/ml. Sixty-three percent of these patients had prior regular cycles, and 50% had previously been pregnant. The patient with empty-sella syn-

drome presented with amenorrhea and galactorrhea after 6 term pregnancies; the diagnosis was made before the availability of prolactin assays.

Seventy-three patients presented with chronic anovulation of the polycystic ovarian disease type. Fifty-five percent of these women had prior regular cycles, and 24% of them had previously been pregnant.

Postpill amenorrhea. Seventy-seven (29%) of all patients with adult-onset amenorrhea could be labeled as having postpill amenorrhea (Table IV). Nearly half (48%) of these patients had some form of hypothalamic suppression, and over half of these hypothalamic-suppressed women had associated weight loss. Other diagnoses made in the group with postpill amenorrhea included ovarian failure (10 patients), hyperprolactinemia (13), Asherman's syndrome (two), and chronic anovulation (15). Sixty-nine percent of all postpill patients previously had regular cycles. Prior menstrual rhythm did not differ appreciably between these groups represented (Table III).

Two patients were diagnosed as having hyperprolactinemia while being treated with estradiol pellets. They had the highest prolactin values of any of the hyperprolactinemic patients (1600 ng/ml and 1500 ng/ml, respectively), and both had macroadenomas as diagnosed by computerized tomographic scans.

Comment

This is the largest series of patients presenting with secondary amenorrhea published from a single institution. Previous studies of amenorrhea patients have been directed toward the examination of primary amenorrhea alone,^{3,4} of a combination of primary and secondary amenorrhea,⁵⁻⁷ or of secondary amenorrhea alone.⁸ These studies have often been directed toward determining the usefulness of certain diagnostic tests for categorization of such patients rather than the understanding of the relative frequency of occurrence of specific disorders and their manner of clinical presentation.⁶⁻⁸ The present series of 262 patients was reported in an attempt to study a specific group of secondary amenorrhea patients: those women in whom the etiology for amenorrhea was largely independent of abnormalities of the pubertal process. Women were excluded who developed secondary amenorrhea within six cycles or 6 months of menarche. Those patients whose secondary amenorrhea had developed in and around the pubertal process were included in a group of 252 patients with pubertal amenorrhea (primary or secondary) studied during this same time period at the same institution and previously reported.² This present series of women with adult-onset amenorrhea is reported to contrast their clinical picture with that of patients with pubertal amenorrhea. The facts that the average age of menarche for all patients was

12.87 years and that the average age of presentation was 26.4 years adds support to the adult-onset nature of the present series.

Table II reveals that the etiologies for adult-onset amenorrhea differ significantly in frequency of occurrence from those of pubertal amenorrhea.² In the prior series the three most common etiologies identified were ovarian failure (43%), congenital absence of the uterus and vagina (15%), and physiologic delay of puberty (14%).² In contrast, the present series identified the four most common causes of amenorrhea in the adult years as being hypothalamic in origin (33.5%), as chronic estrogenic anovulation (28%), as hyperprolactinemia (14%), and as ovarian failure (12%). The stresses of the adult years seem to take their toll on the hypothalamus and pituitary and represent the most common cause of adult-onset amenorrhea. Half of the patients in this series with hypothalamic amenorrhea had reported weight loss. Many of the other women in this group reported potential causes of hypothalamic-pituitary suppression such as stress, exercise, or systemic illness. Women with an estrogenic form of amenorrhea who responded to a progestin challenge and who did not have an identifiable etiology were considered to have chronic anovulation (Table II). A number of these women presented with the clinical picture which could be labeled as polycystic ovarian disease. It could be argued that this group of women has an etiology which is dependent on an aberrancy of pubertal maturation and is not of adult onset. The pubertal amenorrhea series included 7% of all patients with typical polycystic ovarian disease having primary or secondary amenorrhea that developed in and around puberty. In the present series 55% of women with chronic anovulation had prior regular cycles. This demonstrates the likely heterogeneity of the syndrome of chronic anovulation. Eight of 37 women with hyperprolactinemia also had estrogenic anovulation. This suggests that in some women hyperprolactinemia may be the result of the same neuroendocrine mechanism that is responsible for the polycystic ovarian disease state or that early developing hyperprolactinemia may mimic chronic anovulation. These data in addition to the fact that hyperprolactinemia represents the third most common etiology for adult-onset amenorrhea provide support for the common practice of obtaining a serum prolactin determination in any woman with amenorrhea. Both of the series of amenorrhea studied at the Medical College of Georgia demonstrate that the premature loss of ovarian follicles most commonly manifests itself clinically as ovarian failure during the normal time period for pubertal onset. Women who develop ovarian failure at times more removed from puberty are less likely to have a form of Turner's syndrome. However, the present series further demon-

strates that Turner karyotypes may be identified in amenorrheic women even after age 35 years.

This series, like prior reports,^{7,8} classifies patients according to gonadal function (Table I). Nearly half of studied patients (54%) were hypogonadal on presentation and the other half (46%) were eugonadal. Use of this classification will identify patients who are clearly at risk for ovarian failure (hypoestrogenic) and others with a potential target organ failure (estrogenic). However, a significant overlapping of etiologies occurs between the hypogonadal and eugonadal groups. Tables II and III demonstrate that nonspecific hypothalamic patients and women with hyperprolactinemia may be either hypoestrogenic or estrogenic on presentation. All of the patients identified in this series with hypothyroidism, Cushing's syndrome, and adult-onset congenital adrenal hyperplasia were hypoestrogenic. Hypothyroidism has previously been described to alter menstrual function. It rarely produces amenorrhea and its impact on gonadal function is variable.⁹ Cushing's disease may similarly present with either hypogonadism or eugonadism. Congenital adrenal hyperplasia of adult onset may present not unlike polycystic ovarian disease. Because congenital adrenal hyperplasia is not easily identified in the adult years, a number of such patients in our series may have been misdiagnosed as having chronic estrogenic anovulation.

The manner of presentation of women in this series did not differ significantly. The average age of presentation for all patients was 26.4 years. Mean ages from category to category ranged from 21 to 30 years, with a few patients reported separately as being 18, 19, and 38 years of age. The group of four patients with Sheehan's syndrome had the oldest mean age of presentation, 31.8 years. Hyperprolactinemia patients in category 2 also presented at the older age of 30 years. The youngest groups were patients with weight loss amenorrhea at 21.9 years and those with inappropriate positive feedback at 22 years. Duration of amenorrhea before presentation varied remarkably between the groups. Historic information gathered for this series demonstrates that pregnancy had previously occurred in all patients with Sheehan's syndrome and Asherman's syndrome. It occurred in nearly half of all patients reported with chromosomally incompetent ovarian failure, nonspecific hypothalamic stress, hypothyroidism, and ovarian tumors and in categories 3 and 5 with hyperprolactinemia. It occurred in less than one third of the patients with chromosomally competent ovarian failure, weight loss amenorrhea, estrogenic nonspecific hypothalamic amenorrhea, hyperprolactinemia categories 1, 2, and 4, and inappropriate positive feedback. The one patient with combination hypothyroidism and hyperprolactinemia, both patients with Cushing's syndrome, the one patient with an undi-

agnosed hypoenestrogenic hyperandrogenic state associated with diabetes mellitus, and the patient with an unclassified tumor had never previously achieved pregnancy. Although the data collection process used for this study did not separate out elective abortions from spontaneous abortions, the data of total pregnancy loss revealed interesting trends. Eighty-five percent of pregnancies for the two Turner patients who achieved pregnancy were lost. This is not unexpected inasmuch as it is a known fact that aneuploidic individuals are at significant risk for conceiving aneuploidic progeny. The gestational loss of 36% for women with Asherman's syndrome is also not unexpected. Pregnancy loss rates approximated that of the general population for women with chromosomally competent ovarian failure, hyperprolactinemia, and Sheehan's syndrome. Unexpected high pregnancy loss rates were reported for patients with weight loss/anorexia (43%), women with hypoenestrogenic nonspecific hypothalamic suppression (35%), women with estrogenic nonspecific hypothalamic suppression (70%), and others with inappropriate positive feedback or chronic estrogenic anovulation (32%). Although it was not possible to separate elective abortions from the spontaneous abortions for this study and although the total numbers of pregnancies was small in each group, these data suggest that there is a significant fetal mortality risk when conceived by women with estrogenic or hypoenestrogenic forms of anovulation not associated with hyperprolactinemia. Further studies of this group of women are indicated to understand the exact risk of these patients for recurrent spontaneous abortions.

Postpill amenorrhea occurred in 77 (29%) of all women studied (Tables III and IV). Diagnoses given to these women were found in each of the major categories represented by adult-onset amenorrhea (Tables III and IV). It has often been said that patients who develop amenorrhea after discontinuing the oral contraceptives were usually irregular before starting use of oral contraceptives. However, at least 60% of all women with postpill amenorrhea in the present series had prior regular cycles (Table IV). A number of investigators have examined the potential cause-and-effect relationship between oral contraceptives and the development of certain disorders that result in amenorrhea. It is difficult to incriminate the oral contraceptives for causing ovarian failure or chronic estrogenic anovulation. In our series the atrophic effect of oral contraceptives on the endometrium of two patients combined with a prior obstetric problem in one woman and a dilatation and curettage while experiencing oral contraceptive amenorrhea in the other makes a cause-and-effect relationship likely. Investigators have produced data that make the oral contraceptives highly suspect for causing amenorrhea in two other groups of

Table IV. Postpill amenorrhea

	No. of patients	%	Prior regular cycles	
			n	%
Chromosomally competent ovarian failure	10	13	6	60
Weight loss amenorrhea	20	26	13	67
Nonspecific hypothalamic	17	22	14	81
Hyperprolactinemia	13	17	8	62
Asherman's syndrome	2	3	2	100
Chronic anovulation	15	19	10	66
Total	77	100	53	69

patients: women with weight loss amenorrhea and those with hyperprolactinemia.

In 1973 Fries and Nillius¹⁰ reported the association of weight loss and dietary restriction with oral contraceptive usage. Forty-five of 75 patients with postpill amenorrhea in the study admitted to weight gain during oral contraceptive usage and most commonly during the first 6 months. Fifteen percent of these women gained more than 5 kg. Forty-five percent of patients with postpill amenorrhea admitted to a deliberate dietary restriction while using oral contraceptives, with an average weight loss of 9.3 kg. Although most women began dieting during oral contraceptive treatments, 25 women continued dieting after discontinuing their use. These authors concluded that one of the most feared side-effects of women using oral contraceptives was weight gain and that the deliberate weight reduction either while using them or shortly after stopping therapy was an important cause of postpill amenorrhea. Hancock et al.¹¹ studied 64 patients with postpill amenorrhea, 29 of whom had prior regular cycles and 27 who were previously irregular. The patients who were regular before initiating oral contraceptive usage had a significantly lower mean body weight than either those women who had been irregular before or the 100 control subjects. These authors concluded that "in the absence of pre-existing ovulatory dysfunction, a low body weight, not necessarily associated with recent weight loss, is of major significance in development of postpill amenorrhea." In a study of 24 women with primary or secondary amenorrhea, Wentz¹² found that patients using oral contraceptives needed to lose only minor degrees of body weight or fat for the development of amenorrhea as compared to other women with weight loss amenorrhea not taking the oral contraceptives. Nearly 50% of patients with postpill amenorrhea in our series had hypothalamic amenorrhea, and one half of those patients had reported an associated weight loss (Table IV). Sixty-seven percent of the weight loss

patients with postpill amenorrhea and 1% of the non-specific hypothalamic suppression women with postpill amenorrhea had prior regular cycles. Although none of the above data is conclusive, it all suggests that women using oral contraceptives who are of reduced body weight may be at an increased risk for the development of postpill amenorrhea should they significantly reduce caloric intake or perhaps increase caloric expenditure either while using oral contraceptives or during the immediate posttherapy period.

The association between oral contraceptives and the development of hyperprolactinemia has been of concern to a number of investigators. Estrogen has been shown to increase messenger ribonucleic acid for prolactin production. In experimental rodent models, adenomatous changes within the pituitary can be produced by high levels of estrogen. In 1977 a case report of the development of a microadenoma associated with gynecomastia, galactorrhea, and impotence in a man with prolonged industrial exposure to mestranol and norethindrone by inhalation aroused concern that prolonged oral contraceptive usage might produce prolactinoma formation in humans. Several investigators have demonstrated that a large portion of women with surgically proven pituitary tumors had reported a prior history of oral contraceptive usage.^{13, 14} In one series 74% of 42 women with proven adenomas developed amenorrhea and/or galactorrhea in immediate association with the use or discontinuation of oral contraceptives or immediately post partum.¹⁵ However, there are similar prospective and case-control studies that show no cause-and-effect relationship.^{15, 16} In the present series 48% of all patients with hyperprolactinemia had postpill amenorrhea and 17% of women with postpill amenorrhea had hyperprolactinemia. Sixty-two percent of the hyperprolactinemic women with postpill amenorrhea had prior regular cycles. The two highest prolactin values of 1600 and 1500 ng/ml were reported in women who had been given estradiol pellets over at least 4 years. Oral contraceptives probably do not induce hyperprolactinemia in women. However, in some patients at risk the long-term usage of pharmacologic doses of hormones may stimulate galactroph hyperplasia at a faster rate than would otherwise have occurred. The accumulative data suggest that it may be beneficial to obtain prolactin levels in any patient who begins using oral contraceptives in the setting of a menstrual irregularity and in all patients at subsequent intervals during prolonged oral contraceptive usage. Additional prospective studies are in order to understand better the relationships between body weight and amenorrhea as well as oral contraceptive usage and development of hyperprolactinemia.

Both of the Medical College of Georgia studies have

examined the quality of life for these patients in the face of optimal treatment.² The pubertal amenorrhea series reported the identification of tumors that were largely gonadal in patients with XY cell lines. Morbidity and mortality occurred infrequently in the pubertal amenorrhea individuals. Most importantly, the prior study reported a significant reduction in reproductive potential for patients with pubertal aberrancy. Nearly 60% of those patients had absolute barriers for reproduction and only 14% of them had normal reproductive potential. Adult-onset amenorrhea appears to present less significant detrimental effects on the quality of life for women than does pubertal amenorrhea. The potential for gonadal tumors is no greater for women with secondary amenorrhea of any etiology than it is for women of the general population providing that these women have a normal female phenotype. The most common tumors identified in this series were pituitary in origin ($n = 10$), and these probably do not represent true neoplasias. Morbidity was most significant for the four women with panhypopituitarism (Sheehan's syndrome). Mortality did not occur but is most possible in the group of women with anorexia nervosa. Virtually all women with adult-onset amenorrhea, including the Turner patients, had the potential for reproduction before the onset of amenorrhea. Absolute barriers for reproduction occur in only the 12% of women identified with ovarian failure. A small portion of the 7% of women with Asherman's syndrome may also have an absolute barrier to fertility. Fifteen percent of the patients diagnosed with 46,XX ovarian failure subsequently became pregnant.

In summary the terms primary and secondary amenorrhea provide little delineation between potential diagnoses or significance for a given patient. The data from the series of patients with pubertal aberrancy previously reported and from the present study demonstrate that far more differences exist between pubertal amenorrhea and adult-onset amenorrhea. Although ovarian failure is the most common diagnosis made for the former group, hypothalamic suppression will be seen most frequently in the latter period. Congenital abnormalities of the central nervous system and genital tract will be limited to pubertal amenorrhea. Acquired abnormalities of the central nervous system and genital tract will become more evident in the adult years. Adult-onset amenorrhea presents a far more benign entity than does pubertal amenorrhea. Morbidity and mortality may occur in either group. Although reproductive potential is most affected by the label of pubertal amenorrhea, patients in both groups may have significant difficulty in achieving pregnancy. Early diagnosis, treatment, and counseling remain essential for all patients with amenorrhea.

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Discussion

DR. ALLAN G. W. MCLEOD, Miami, Florida. The authors have provided us with an extensive and impressive review of 262 cases of adult-onset amenorrhea seen over a 20-year period at the Medical College of Georgia, outlining the relative frequency of specific disorders and grouping patients according to estrogen status.

Changes in methods of investigation over a 20-year span may have had a bearing on the accuracy of the diagnosis in earlier cases. It was of particular interest that the authors were able to compare this series with another large impressive study from the same center of a group of women with amenorrhea of pubertal onset.

Although the review is informative and even at times

a little confusing because of the wealth of information it contains, the authors do not appear to be introducing any truly new concepts. I believe their conclusions are similar to those already reflected in the literature.

A number of points should be emphasized. The use of skull films with coned-down view of the sella turcica was discussed. At present, when a high prolactin level is found, a third-generation computerized tomographic scan is to be preferred to avoid missing a small prolactinoma.

The patient with an abnormal karyotype should be studied for associated anomalies. Did this subgroup of women have intravenous pyelography performed?

Five categories of women with hyperprolactinemia were outlined. One should remember that the actual value of the serum prolactin may be less important than the fact that it is elevated. Pituitary tumors have been reported with prolactin levels well below the stated 100 ng/ml. It is also wise to confirm elevation of the serum prolactin before instituting expensive radiologic studies.

The concept of a regular cycle range being 21 to 40 days in a group of women with an average age of 26 years raises the question of whether or not women with polycystic ovaries and anovulatory cycles may have been included.

It should also be noted that patients described as having chromosomally incompetent ovarian failure had experienced prior regular cycles and some of them had achieved a pregnancy. Consequently it should be noted that the maternal stigmas of hypogonadotrophic hypogonadism are not necessarily associated with infertility.

The chromosomally competent ovarian failure of some women subsequently resolved; were these examples of an ovarian resistant syndrome and were these women given any specific treatment?

A 29% incidence of postpill amenorrhea seems very high. Did most of these cases occur earlier in the study period when higher doses were used? Attention should also be given to the association of postpill amenorrhea and weight loss in almost half of this group of women with hypothalamic amenorrhea. It should also be noted that 60% of the author's group had regular cycles before the use of an oral contraceptive. A useful discussion of the relationship to weight loss and previous menstrual pattern outlining the significance of mean body weight and weight reduction while on the pill or shortly after discontinuance is presented. Certainly attention should be given to the suggestion that reduction of calorie intake in women of low body weight while using oral contraceptives or during the immediate post-treatment period may result in postpill amenorrhea.

The relationship between hyperprolactinemia and postpill amenorrhea was interesting as was the fact that 62% of these women had regular cycles. The suggestion that it may be beneficial to obtain serum prolactin levels in women with irregular menstrual cycles who are about to commence using an oral contraceptive and that this

test should be repeated at regular intervals during prolonged oral contraceptive usage merits attention.

Two patients receiving treatment with estradiol pellets were described as having extremely high prolactin levels and were subsequently found to have microadenomas. Were they receiving this treatment for a surgical or natural menopause?

The authors' studies demonstrate that women with adult-onset amenorrhea have a distinctly better prognosis with regard to fertility than those with the pubertal type and that their potential for gonadal tumors is not increased provided they are phenotypically normal.

I still have a preference for the terms primary and secondary amenorrhea as opposed to pubertal and adult-onset and feel that the presence of prior menses does provide important clinical information and rules out some of the causes of amenorrhea, for example, müllerian agenesis and testicular feminization.

In conclusion, the authors state that the main causes of adult-onset amenorrhea are hypothalamic origin (33.5%), chronic estrogenic anovulation (28%), hyperprolactinemia (14%), and ovarian failure (12%); however, after 25 years at Jackson Memorial Hospital with 11,800 deliveries a year I am convinced that they all run a poor second to *pregnancy*!

DR. FRANK G. RIGGALL, Gainesville, Florida. Dr. Reindollar has presented a large retrospective series, collected over 20 years at the Medical College of Georgia, of patients less than 40 years old who presented with secondary amenorrhea of at least 6 months' duration, following at least six menses after menarche. These patients are then contrasted with 252 patients with developmental delay from the same institution reported previously. He found hypothalamic pituitary problems to predominate in the adult-onset group whereas gonadal and genital tract abnormalities were most common in the latter group.

It seems to me there is little difference between use of the conventional categories primary and secondary amenorrhea versus pubertal and adult-onset amenorrhea, since all patients with primary amenorrhea will fall into the pubertal category and most patients with secondary amenorrhea will fall into the adult-onset category. Certainly, primary and secondary amenorrhea are less cumbersome terms and are easily understood. Both types of amenorrhea require nearly identical evaluation, although the etiology of the amenorrhea is more likely to be related to anatomic or chromosomal problems in the pubertal or primary group and more often to inappropriate feedback in the secondary amenorrhea or adult-onset group. Complete evaluation is essential in both groups. Indeed the only category in the pubertal group which was not represented in the adult-onset group were those with anatomic defects.

Nearly one third of the patients in the present study noted the onset of their amenorrhea following discontinuation of oral contraceptives; however, only half of this group had amenorrhea related to hypothalamic suppression. It is noteworthy that 50% of the postpill amenorrhea group had regular menses before oral con-

traceptive use. The authors suggest that weight loss during or following use of oral contraceptives may predispose women to amenorrhea, but this group, in fact, encompassed only one quarter of the total. The remainder of the group was divided nearly evenly between patients having chronic anovulation, nonspecific hypothalamic causes, and hyperprolactinemia.

Only 38 of the study patients had hyperprolactinemia, although it is unclear how many patients underwent prolactin determination. Previous reports have suggested that up to 30% of patients with amenorrhea may have hyperprolactinemia and 50% of those with amenorrhea/galactorrhea may be hyperprolactinemic. The authors do not mention the presence or absence of galactorrhea, which serves as a useful marker in about one third of patients with hyperprolactinemia. While no cause-and-effect relationship has been proved to exist between oral contraceptive use and subsequent prolactinoma development, it is interesting that two patients treated with estradiol pellets had levels of prolactin which exceeded 1000 ng/ml and both of these patients were found to have tumors. The overall incidence of pituitary tumors among the hyperprolactinemic group was 32% and may have been higher if computerized tomographic scanning had been available for use in all patients. Indeed the incidence of pituitary adenomas had been reported to be as high as 27% in a random autopsy series.¹

Dynamic testing with insulin, gonadotropin-releasing factor, adrenocorticotrophic hormone, and thyrotropin-releasing factor is mentioned, but no data are provided in the paper as to the results of such tests. Do the authors find these tests useful routinely? Our experience at the University of Florida has been that the current generation computerized tomographic scan is more sensitive than routine hormonal testing in detecting microadenomas.

The four patients with chromosomally incompetent ovarian failure remind us that pregnancies are possible among Turner's syndrome patients. Although Turner mosaics are more likely to have menses and pregnancies, 11 patient with 45,X karyotype have been reported in the literature with documented pregnancies.²

Of the 27 patients with chromosomally competent presumptive ovarian failure, three had resolution of amenorrhea and resumption of menses. Rebar et al.² reported evidence of follicular activity in nine of 18 women with follicle-stimulating hormone levels >40 mIU/ml who had daily blood determinations for gonadotropin and estradiol levels. He suggested weekly determinations of estradiol, luteinizing, and follicle-stimulating hormone be done for 1 month before concluding that ovarian failure was indeed present. Estrogen replacement therapy has been associated with resumption of ovulation and even pregnancy in patients with presumed ovarian failure. Estrogen is known to increase the number of follicle-stimulating hormone receptors in ovarian granulosa cells, thus making them more responsive to the actions of follicle-stimulating hormone. Autoimmune disease should be searched for in patients with chromosomally competent ovarian fail-

ure, and levels of calcium, phosphorus, thyroid function and antibodies, and adrenal reserve should be assessed. Did patients in this group receive estrogen replacement and were they screened for abnormalities of other endocrine organs?

This paper tabulates and describes the many nuances of the clinical presentation of secondary amenorrhea and serves as a reminder that evaluation of this multifaceted condition should be meticulous and complete.

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DR. ROBERT A. KNUPPEL, Tampa, Florida. Has anybody had experience with the use of magnetic resonance imaging in the imaging of the sella turcica of the pituitary area and with that enhanced diagnosis?

DR. JOHN B. HILL, Athens, Georgia. I would like to ask Dr. Reindollar whether he is doing any antinuclear factor or lupus erythematosus preparation on these patients that have premature menopause or premature onset of amenorrhea?

DR. H. OLIVER WILLIAMSON, Charleston, South Carolina. Both of the official discussants touched on one subject, but I would like to put it a little more bluntly. Did these hypergonadotrophic patients who had spontaneous resolution have biopsies? The discussants have already raised the question, Were they treated with hormones?

DR. REINDOLLAR (Closing). To begin with, our study is certainly not a prospective study, and it is not a case-control study. Rather, it is a description of a vast experience with patients having amenorrhea which occurred during a 20-year time period when a great number of changes occurred in both diagnosis and management. Therefore there were several inherent problems with our study. At the same time I think it provided an experience that would be hard to duplicate in a prospective fashion today.

In addressing Dr. McLeod's concerns and comments, first he said that during this time period, the 20 years, perhaps there were some diagnoses that were not appropriately made, and indeed, that is accepted by our study. For instance, it is very possible that some patients with adult onset congenital adrenal hyperplasia were misdiagnosed as having a form of chronic anovulation. He also asked whether intravenous pyelography was performed in patients with chromosomal incompetency; yes, they were in all these patients. In our prior series of Turner patients up to 30% had cardiovascular or renal abnormalities. We did not find renal abnormalities in this present group. Dr. McLeod asked whether the cycle lengths of 21 to 40 days, which were considered regular cycles, may have included some pa-

tients with polycystic ovarian disease. It is certainly possible. There are studies in the literature which demonstrate that ovulatory cycles may range from 21 to 40 days. However, in our series we were only studying patients who had 6 months of amenorrhea or more.

There were a number of questions concerning the patients with chromosomally competent ovarian failure who had resolution of ovarian failure. This certainly has been reported in the literature before. All of our patients had gonadotropin assays. There were three who had FSH levels >50 mIU/ml on several occasions, and these patients were treated with estrogen. We did not have ovarian biopsies of them or laparoscopic visualization of their gonads as asked by Dr. Williamson. Regarding the postpill amenorrhea and the question of whether 29% is a high incidence, this is similar to other studies that are reported in the literature.

Dr. Riggall addresses several concerns: first, he believes that perhaps there is no benefit in using the terms adult-onset amenorrhea and pubertal amenorrhea rather than primary and secondary amenorrhea. Perhaps he is correct. At the same time I believe these terms may provide at least a bit more description about the time period when the amenorrhea began. We know that in the pubertal group there were both primary and secondary amenorrheas. Also, there appears to be a much worse prognosis associated with pubertal amenorrhea than is associated with amenorrhea of adult onset. Referring to the postpill amenorrhea group, he notes that only a fourth of them had weight loss. That is true, but again it brings out the idea that perhaps a reduced body weight or loss of weight while using oral contraceptives may increase the risk for postpill amenorrhea.

There were several questions in regard to prolactin determinations. First was the fact that only about 30 of our patients were hyperprolactinemic. All of the patients were seen before 1980. We have certainly a number of additional hyperprolactinemic more patients that have been referred in after the 1980s, and if we looked at this series now, perhaps these would represent a larger proportion of the total series. In this particular study, we did not ascertain from our patients the presence or absence of galactorrhea, so I cannot give you that information. The prolactin studies were obtained on all patients after the mid-1970s, when prolactin assays became available. We also mentioned the fact that dynamic studies were performed in some of these patients. With the evolution of diagnostic abilities through the years, it became apparent to us that dynamic studies, at least for the prolactin group, are perhaps rarely helpful.

Dr. Knuppel asked whether or not we used magnetic resonance imaging. The medical college recently acquired an imager with which we do not yet have experience. In response to Dr. Hill's question, we do evaluate all patients with chromosomally competent ovarian failure for autoimmune disease processes, looking for thyroid antibodies, and also evaluate for such things as ANA. It was only in the more recent years that we have been sending out our blood samples for ovarian antibody studies.

Effect of maternal-fetal disorders on lung maturation

I. Diabetes mellitus

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Amniotic fluid for fetal lung maturity studies was obtained from 287 healthy and 198 diabetic women. Classes of diabetes were as follows: Class A, 111; Class B, 58; Class C, 13; Class D, 11; Class F, 4; and Class R, 1. The regression lines representing the relationship of amniotic fluid lecithin phosphorus concentration to gestational age at amniocentesis in each of the groups of diabetic patients were not statistically different from those of the control subjects. Each of the diabetic patients was then matched with a control subject of the same race, sex of newborn infant, and gestational age at amniocentesis. The regression lines of the nonhypertensive, hypertensive, and all diabetics were not different from those of their respective matched control subjects. Also there was no difference in the proportion of mature lecithin phosphorus concentrations at different weeks between diabetic and normal women. The absence of a significant influence of diabetes on fetal lung maturation is probably due to improvement in diabetic control resulting in normalization of the fetal metabolic environment. (AM J OBSTET GYNECOL 1986;155:544-53.)

Key words: Fetal lung maturity, normal pregnancy, diabetes mellitus, amniotic fluid lecithin

The improved outcome of diabetic pregnancies depends in a large measure on accurate timing of delivery. This is usually determined by consideration of the degree of metabolic control, tests of fetal well-being, and documentation of fetal lung maturity.¹ An effect of diabetes mellitus on fetal lung maturation has been suggested by both animal experiments and clinical observations based on assays of amniotic fluid surfactant.²⁻⁷ Most of the latter used the lecithin/sphingomyelin ratio as the test for fetal lung maturity. The earlier investigations generally suggested delay of lung maturation in "uncomplicated" diabetes and acceleration in diabetes complicated by vascular disease,² but there were disagreements about the effects in individual classes of diabetes, while some investigations failed to demonstrate an effect in any of the classes.³ On the other hand, the more recent investigations have generally failed to show an effect of diabetes mellitus on the lecithin/sphingomyelin ratio.⁴⁻⁶ This method depends on the amniotic fluid content of sphingomyelin, a non-surface-active phospholipid. This introduces another variable that by itself is not related to fetal lung maturation.

This may, at least in part, explain the variable results cited.

Other indices of fetal lung maturity have also been used to study the effect of diabetes mellitus, for example, the measurement of amniotic fluid lipid extract surface tension, the measurement of amniotic fluid microviscosity with the use of fluorescence polarization, the assay of amniotic fluid phosphatidylglycerol,^{4, 6, 7} phosphatidylinositol,^{4, 6} disaturated phosphatidylcholine, or the "lung profile."⁸

In our institution, we have used the measurement of acetone-precipitable lecithin (expressed as its phosphorus content) as the fetal lung maturity test.⁵ The amniotic fluid lecithin phosphorus concentration of ≥ 0.1 mg/dl is considered indicative of maturity in both normal and abnormal pregnancies, including diabetic pregnancies.^{1, 8, 9}

We are reporting the effects of diabetes mellitus on the maturation of the fetal lung as determined by this test.

Material and methods

Included in this study are pregnant women who were managed and delivered at the Medical College of Georgia Hospital during the years 1979 to 1984. The study includes both normal and diabetic third-trimester pregnant women. All patients had undergone at least one transabdominal amniocentesis for fetal lung maturity assessment as part of their management.^{1, 9} Patients with abnormal hemoglobins, other medical disorders (excluding those that are related to diabetes), multiple

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pregnancy, placenta previa or abruptio placentae, or isoimmunization and those who had premature rupture of the membranes (before amniocentesis) or received antenatal glucocorticoids were excluded from the study. In the normal group the majority (80%) of amniocenteses were performed before a repeat cesarean section. Most of the remainder were done for determination of fetal lung maturity in patients with unknown menstrual dates or suspected postdatism. The great majority of women included in the normal group also had had a normal glucose screening test and had been delivered of normal, healthy, adequate for gestational age babies at term.

All amniocenteses were done under real-time ultrasonic guidance. The amniotic fluid samples that were meconium or blood stained were excluded from the study. The lecithin phosphorus concentration was measured in 10 to 20 ml aliquots of amniotic fluid, according to a previously described method.⁸

The gestational age at delivery was based on menstrual dates and confirmed in the majority of cases by ultrasonic dating. Neonatally assessed gestational age was within ± 2 weeks of the estimated gestational age in all cases in the study.

The maternal age, race, gravidity, parity, gestational age at delivery, birth weight, sex of the infant, and dates of amniocentesis and delivery as well as the results of amniotic fluid lecithin phosphorus concentrations were entered into a computer. The interval between amniocentesis and delivery was computed in weeks or fractions thereof and the gestational age at amniocentesis was calculated. In the diabetic patients, the class of diabetes as well as the presence or absence of hypertension was also entered. The data were analyzed with the Statistical Package for the Social Sciences. Tests used included Student's *t* test, Pearson's correlation, analysis of variance, Tukey's multiple comparisons-HSD (honestly significant differences procedure), multiple regression, covariance analysis, and χ^2 test.

Results

The number of patients in the study and the distribution of the diabetic patients according to White's classification is presented in Table I. Because of the few number of patients in Classes F (four) and R (one), they were added to Class D (11) patients. Also shown in Table I is the number of amniotic fluid samples studied in each of these groups of patients. The racial distribution of the normal and diabetic patients was very similar; the majority were black (Table II). Some pertinent clinical data of the study patients are shown in Table III. The diabetic patients were significantly older and were of higher gravidity and parity than the control subjects. The mean gestational age at delivery was slightly less and the birth weight was slightly more in

Table I. The study patients and amniotic fluid samples

	No. of patients	No. of samples
Normal	287	313
Diabetic	198	247
Class A	111	126
Class B	58	78
Class C	13	17
Classes D, F, and R	16	26
Total	485	560

Table II. Race

	Normal		Diabetic*	
	No.	%	No.	%
Black	182	63.4	127	64.1
White	98	34.1	70	35.4
Other	7	2.4	1	0.5

*No significant differences (χ^2 test).

the diabetic patient than in the normal women. The sex distribution of the newborn infants was almost identical in both groups (Table IV). The gestational age at amniocentesis is shown in Table V. It was significantly less in the insulin-dependent women than in both Class A diabetic and normal pregnant women. The gestational age at amniocentesis in the individual diabetic classes is presented and compared with gestational age at amniocentesis in the control subjects in Table VI. The gestational age at amniocentesis in Classes D, F, and R was significantly less than that in the control subjects and both Classes A and B. Furthermore, the gestational age at amniocentesis in Class B patients was significantly less than in Class A and normal patients.

The results of amniotic fluid lecithin phosphorus determinations were plotted against the gestational age in all the patients studied. The regression lines representing these relationships are shown in Figs. 1 and 2. As expected, there was a significant positive correlation between amniotic fluid lecithin phosphorus concentration and gestational age at amniocentesis in the normal group, in all individual diabetic classes, in all insulin-dependent diabetic patients, and in all diabetic patients. Compared with the regression line in normal pregnant women, that in Classes D, F, and R appeared to show earlier increase in amniotic fluid lecithin phosphorus concentration with a lower slope. It also appeared that there was a delayed initiation of increase in amniotic fluid lecithin phosphorus concentration in Class C with a higher slope. The initiation of increase in amniotic fluid lecithin phosphorus concentration in Classes A and B and the slope of the respective regression lines were similar to the regression line in the nor-

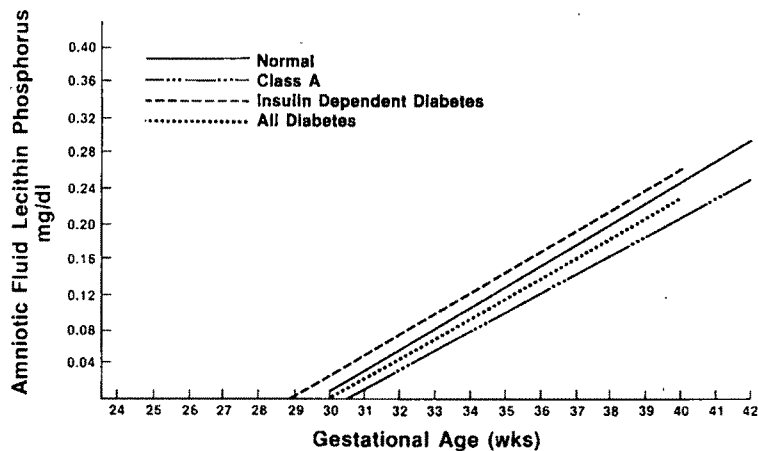


Fig. 1. The relationship of amniotic fluid lecithin phosphorus concentration and gestational age in the study patients. Normal: $Y = -0.69734 + 0.02342X$, $r = 0.342$, $p = 0.00001$. Class A: $Y = -0.69009 + 0.02275X$, $r = 0.356$, $p = 0.00002$. Insulin-dependent diabetic patients: $Y = -0.66288 + 0.02285X$, $r = 0.425$, $p = 0.00001$. All diabetic patients: $Y = -0.64317 + 0.02187X$, $r = 0.375$, $p = 0.00001$.

Table III. Pertinent clinical data of the study patients

	Normal (n = 287)	All diabetic (n = 198)	Class A diabetic (n = 111)	Insulin-dependent diabetic (n = 87)
Age (yr)	23.0 ± 4.9*	28.0 ± 6.8	26.6 ± 6.7	29.7 ± 6.5
Gravidity	2.7 ± 1.2	3.2 ± 2.0	3.1 ± 2.1	3.4 ± 1.9
Parity	1.4 ± 1.0	1.9 ± 1.8	1.8 ± 1.9	2.0 ± 1.7
Gestation at delivery (wk)	38.9 ± 1.6	38.6 ± 2.0	39.4 ± 1.6	37.6 ± 2.0
Birth weight (gm)	3309 ± 474	3362 ± 640	3398 ± 563	3316 ± 729

*Mean ± SD.

Table IV. Sex of newborn infants

	Normal		Diabetic*	
	No.	%	No.	%
Male	163	56.8	112	56.6
Female	124	43.2	86	43.4

*No significant difference (χ^2 test).

mal group. These observations could be interpreted to mean that there is earlier maturation in Classes D, F, and R and delayed maturation in Class C patients. However, when multiple regression was used to compare the regression lines while adjusting for the differences in the gestational age at amniocentesis, the differences between the individual classes of diabetic patients and the normal control subjects were found to be statistically insignificant. Also the differences between all insulin-dependent diabetic patients as well as all diabetic patients and the normal group were statistically insignificant. Moreover, although there seemed to be some differences between the regression lines of the differ-

ent diabetic classes, these differences were not statistically significant.

In order to rule out other possible influences on the maturation of the fetal lung, for instance, race and sex of newborn infant, we matched each diabetic patient with a normal subject whose newborn infant was of the same race and sex and in whom the amniocentesis was performed at the same gestational age (within 0.5 week in the great majority of cases). Diabetic patients with more than one amniotic fluid sample were matched with additional normal patients (one for each additional amniocentesis) at the corresponding gestational age at amniocentesis.

We repeated the analyses and found no statistically significant differences between each of the diabetic classes and their matched control subjects. The same was true for all insulin-dependent diabetic patients and all diabetic patients when compared with their respective matched control subjects (Table VII and Fig. 3).

To examine the effect of hypertensive disease, we divided all diabetic patients into those who had a hypertensive disorder (preeclampsia, chronic hypertensive disease, superimposed preeclampsia, gestational

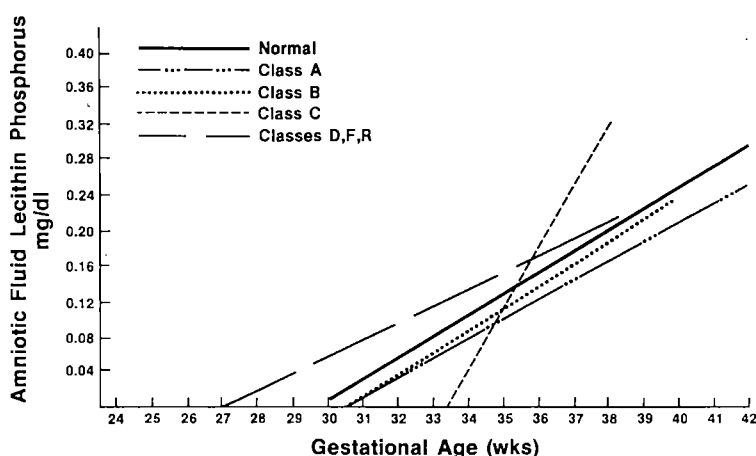


Fig. 2. The relationship of amniotic fluid lecithin phosphorus concentration and gestational age in normal patients and the different classes of diabetic patients. Normal: $Y = -0.69734 + 0.02342X$, $r = 0.342$, $p = 0.00001$. Class A: $Y = -0.69009 + 0.02275X$, $r = 0.356$, $p = 0.00002$. Class B: $Y = -0.75454 + 0.02513X$, $r = 0.394$, $p = 0.00002$. Class C: $Y = -2.27311 + 0.0679X$, $r = 0.168$, $p = 0.004$. Classes D, F, and R: $Y = -0.47632 + 0.01763X$, $r = 0.487$, $p = 0.007$.

Table V. Gestational age at amniocentesis in normal and diabetic patients

	No. of amniocenteses	Weeks	
		Mean $\pm$ SD	Range
Normal	313	37.6 $\pm$ 1.9	30.6-42.7
Class A diabetic	126	38.2 $\pm$ 2.2	30.3-41.9
Insulin-dependent diabetic	121	36.3 $\pm$ 2.4*	28.0-38.9
All diabetic	247	37.3 $\pm$ 2.5†	28.0-41.9

Analysis of variance followed by Tukey's-HSD, $p = 0.05$.

*Significantly less than value in both normal and Class A diabetic patients.

†Significantly less than in normal patients.

Table VI. Gestational age at amniocentesis in normal and diabetic patients by class

	No. of amniocenteses	Weeks' gestation (mean $\pm$ SD)	Significant differences*
Normal	313	37.6 $\pm$ 1.9	>Classes B and D, F, and R
Class A	126	38.1 $\pm$ 2.2	>Classes B, C, and D, F, and R
Class B	78	36.7 $\pm$ 2.2	>Classes D, F, and R
Class C	17	36.4 $\pm$ 1.4	
Classes D, F, and R	26	35.1 $\pm$ 3.0	

*Analysis of variance followed by Tukey's-HSD test, $p = 0.05$.

hypertension) and those who did not have hypertension. We then compared the regression lines within Class A, Class B, Class C, and Classes D, F, and R diabetic patients, insulin-dependent diabetic patients, and all diabetic patients (Table VIII and Fig. 4). Again we did not find any statistically significant differences.

There were no statistically significant differences between the proportion of patients with mature lecithin phosphorus values (≥ 0.1 mg/dl) at the different gestational ages between the normal and diabetic patients. Figs. 5 to 7 depict this comparison for Class A, insulin-dependent, and all diabetic patients, respectively.

Comment

Until now, a clear picture of the effect of diabetes mellitus on fetal lung maturation has not emerged.

The advent of amniotic fluid fetal lung maturity tests provided the means for the study of fetal lung maturation in normal and abnormal human pregnancies. The initial report² that fetal lung maturation, as assessed by lecithin/sphingomyelin ratio, is delayed in Classes A, B, and C, while accelerated in Classes D, F, and R, stirred a lot of interest and resulted in a long series of other investigations of the effect of diabetes on fetal lung maturity tests. However, this report was

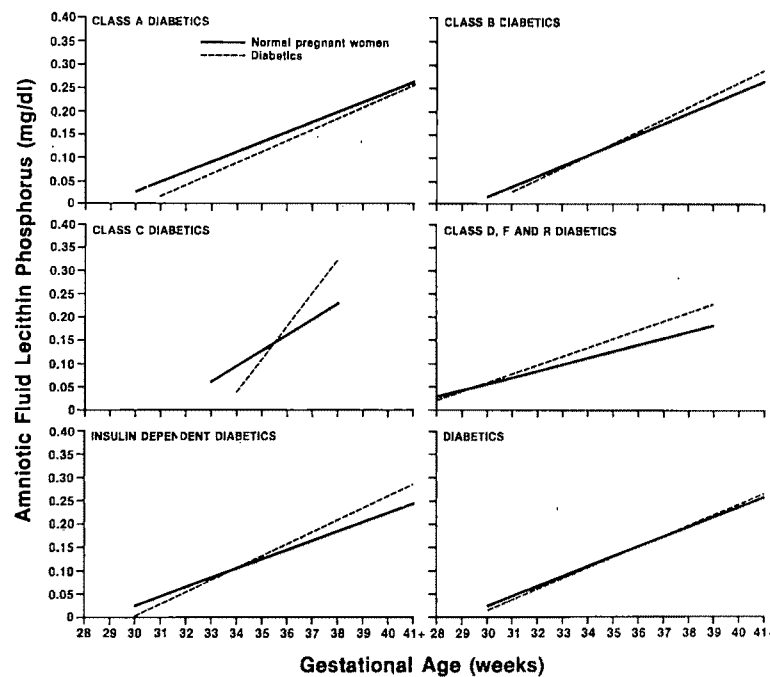


Fig. 3. The relationship of amniotic fluid lecithin phosphorus concentration and gestational age in the diabetic patients and their matched control subjects.

Table VII. Amniotic fluid lecithin phosphorus concentration in diabetic patients and their matched control subjects

	No. of samples	Lecithin phosphorus (mg/dl) (mean $\pm$ SD)	P*
Class A diabetic		0.178 $\pm$ 0.14	
Control	126	0.190 $\pm$ 0.13	0.456
Class B diabetic		0.168 $\pm$ 0.14	
Control	78	0.160 $\pm$ 0.13	0.700
Class C diabetic		0.198 $\pm$ 0.15	
Control	17	0.147 $\pm$ 0.09	0.222
Classes D, F, and R diabetic		0.133 $\pm$ 0.13	
Control	26	0.124 $\pm$ 0.11	0.766
Insulin-dependent diabetic		0.165 $\pm$ 0.14	
Control	121	0.150 $\pm$ 0.12	0.375
All diabetic		0.171 $\pm$ 0.14	
Control	247	0.170 $\pm$ 0.13	0.947

*Paired *t* test.

based on only 33 samples from Classes A, B, and C and only four samples from Classes D, F, and R patients, the gestational age at amniocentesis was not specified, and no statistical comparison was provided. The criteria for exclusion from the normal group were not stated, the samples were contributed from a number of different hospitals, and not all the samples were obtained by amniocentesis. In fact, when larger number of patients were studied and statistical comparison of the regression lines of diabetic and normal patients was performed in a later study, no differences were observed either among the diabetic classes or between any of the classes and the normal pregnant women.⁸ These

results were confirmed in subsequent studies. The proportion of mature lecithin/sphingomyelin ratios at various gestational ages in diabetic and nondiabetic patients was not significantly different.¹⁰ The median lecithin/sphingomyelin ratio in diabetic patients at different gestational weeks (32 to 37) was not significantly different from that in the control subjects.⁹

In our institution, we use the lecithin phosphorus concentration as the fetal lung maturity test.⁵ The main advantage of this test is that it indicates the concentration of the surface-active component (lecithin) without relating it to sphingomyelin, which might be altered in different conditions including diabetes mellitus.¹¹ Ace-

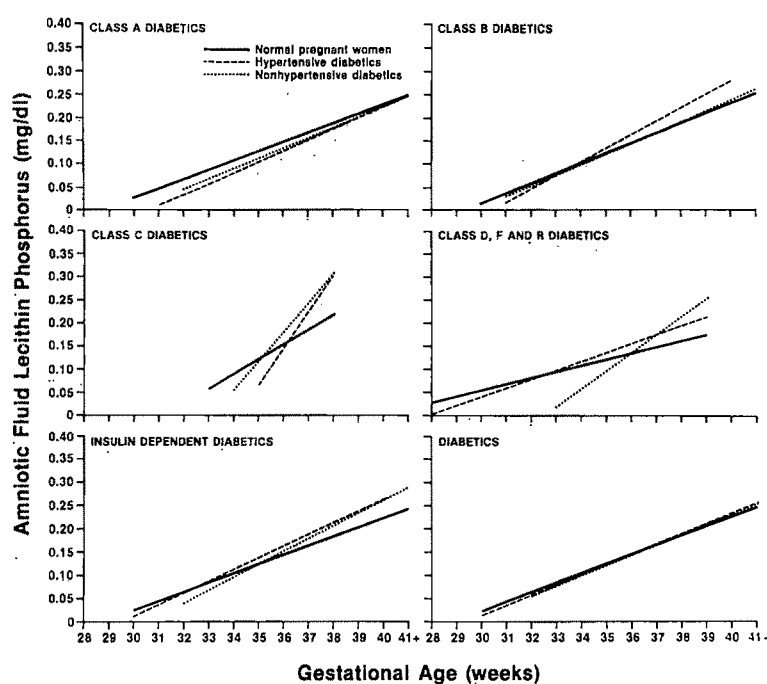


Fig. 4. The relationship between amniotic fluid lecithin phosphorus concentration and gestational age for hypertensive and nonhypertensive diabetic patients and matched control subjects.

Table VIII. Amniotic fluid lecithin phosphorus concentration in hypertensive and nonhypertensive diabetic patients

	No. of samples	Lecithin phosphorus (mg/dl) (mean $\pm$ SD)	P*
Class A diabetic			
Nonhypertensive	89	0.182 $\pm$ 0.14	
Hypertensive	37	0.167 $\pm$ 0.13	0.58
Class B diabetic			
Nonhypertensive	51	0.172 $\pm$ 0.14	
Hypertensive	27	0.160 $\pm$ 0.15	0.73
Class C diabetic			
Nonhypertensive	13	0.210 $\pm$ 0.14	
Hypertensive	4	0.159 $\pm$ 0.18	0.57
Classes D, F, and R diabetic			
Nonhypertensive	10	0.166 $\pm$ 0.15	
Hypertensive	16	0.113 $\pm$ 0.12	0.34
Insulin-dependent diabetic			
Nonhypertensive	74	0.178 $\pm$ 0.14	
Hypertensive	47	0.144 $\pm$ 0.14	0.20
All diabetic			
Nonhypertensive	163	0.180 $\pm$ 0.14	
Hypertensive	84	0.154 $\pm$ 0.14	0.17

*Paired *t* test.

tone precipitation is used because we feel it is important to quantitate only this surface-active component of lecithin (precipitated by acetone) because it is this component that is related to fetal lung maturity. We have found an amniotic fluid lecithin phosphorus concentration of ≥ 0.1 mg/dl to be very reliable in predicting the absence of respiratory distress syndrome in the neonate in both normal and diabetic pregnancies.^{1,8,9}

In this study, we demonstrated a statistically signifi-

cant increase in amniotic fluid lecithin phosphorus concentration with the advancement of pregnancy in both normal patients and all classes of diabetics studied individually or collectively as either Class A, insulin-dependent diabetic patients or as all diabetic patients. It is noteworthy that we used individual points for the construction of these regression lines. All previous authors have averaged the mean value (lecithin/sphingomyelin ratio) at different gestational weeks and then

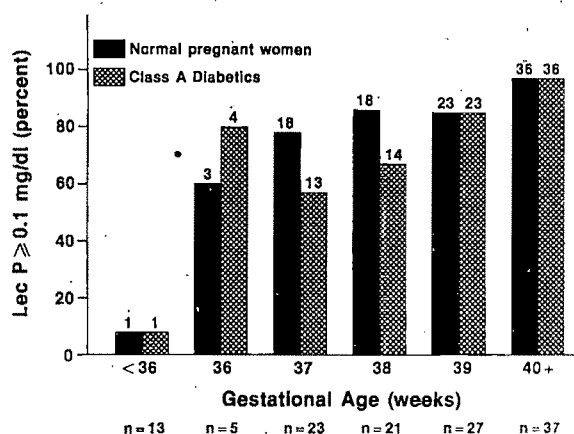


Fig. 5. The incidence of mature lecithin phosphorus concentration in relation to gestational age. Class A diabetic patients compared with their matched control subjects.

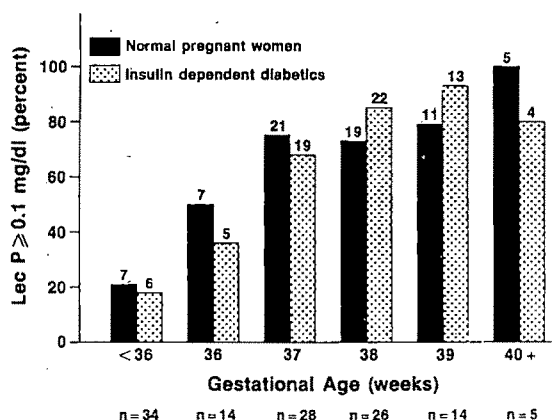


Fig. 6. The incidence of mature lecithin phosphorus concentration in relation to gestational age. Insulin-dependent diabetic patients compared with their matched control subjects.

performed the regression analyses using these means. This tends to "normalize" the data and explains the much higher r value obtained in these studies as contrasted with ours. However, the method we used is more accurate statistically.

Another important advantage of our study is that all the samples were collected from one institution, and all were obtained through transabdominal amniocentesis. All the laboratory determinations were performed under the supervision of a single person and most importantly the criteria for inclusion in the normal group were very strict (see Material and methods). Furthermore, the diabetic patients were managed according to one protocol.⁹

We have shown that there were no differences in the proportion of mature lecithin phosphorus values at different gestational ages between diabetic and normal pregnant women. Also we have shown that the regres-

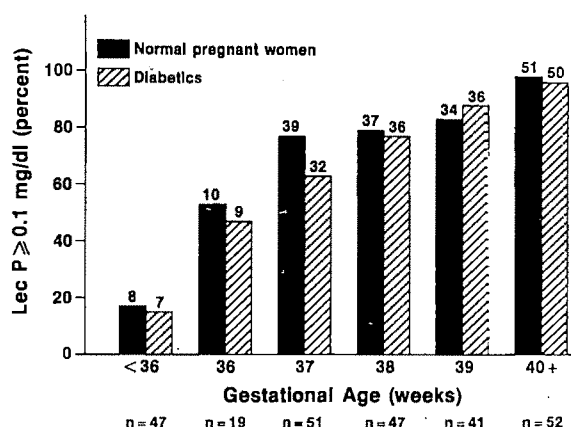


Fig. 7. The incidence of mature lecithin phosphorus concentration in relation to gestational age. Diabetic patients compared with their matched control subjects.

sion lines describing the relationship of amniotic fluid lecithin phosphorus to gestational age in the diabetic patients were not different from those in the normal group.

These results support the absence of a statistically significant effect of diabetes mellitus on lecithin production, in agreement with the previous reports in which lecithin/sphingomyelin ratios were used.^{3-5, 10} The discrepancy between these results and the earlier studies showing such an effect² is probably related to the inclusion of more cases and the use of more accurate statistical designs. However, the improved management of diabetic pregnancies and the recent emphasis on "tight" metabolic control may be important factors. The reported delaying effect of diabetes mellitus was thought to be mediated by fetal hyperinsulinemia. Insulin may interfere with phosphatidylcholine synthesis,¹² alter the availability of glucose to form fatty acids, glycerol, or other substrates, or specifically alter the fatty acid moieties of the lecithin molecule, that is, a decrease of palmitic acid incorporation into phosphatidylcholine to form disaturated phosphatidylcholine.¹² With the strict metabolic control practiced now in our and most other centers, a decrease in the frequency and/or degree of fetal hyperglycemia¹³ and consequently fetal hyperinsulinemia is probably achieved and probably minimized the effects of diabetes on lecithin synthesis and thus on amniotic fluid lecithin phosphorus concentration. This further supports the statement that maternal diabetes may delay normal fetal lung maturation only if it is not properly controlled,¹⁰ a statement that was recently reiterated.¹¹ However, in one study¹⁴ the degree of metabolic control was not found to influence amniotic fluid phospholipids.

An effect of hypertensive disease on fetal lung maturation has been suggested with conflicting results. The interaction between the effects of hypertension and di-

abetes has not been adequately studied. Gabbe et al.¹⁵ could not demonstrate differences in lecithin/sphingomyelin ratios at each gestational age between 21 pre-eclamptic and 189 normotensive insulin-dependent diabetic patients. We have measured amniotic fluid lecithin phosphorus in 84 samples from hypertensive women and 163 from nonhypertensive diabetic women and could not demonstrate a difference in lung maturation between the hypertensive and normotensive groups (Table VIII, Fig. 4).

Several authors have suggested that although lecithin (phosphatidylcholine) synthesis may not be affected in diabetic pregnancies, diabetes may affect the synthesis of the other phospholipids. A significant delay in the appearance of phosphatidylglycerol in Class A (but not in insulin-dependent) diabetic patients was reported by some^{4,6} but not by others.⁷ Phosphatidylglycerol was reported to appear earlier in some Class F and R patients⁴ and in insulin-dependent diabetic patients. However, this earlier appearance in insulin-dependent diabetic patients was not demonstrated in another study⁶ and was reported to be actually delayed in another.⁷ A developmental delay of the anticipated decrease in phosphatidylinositol concentration at 36 weeks in Class A diabetes was noted,⁴ and a lower phosphatidylglycerol/phosphatidylinositol ratio was reported in diabetic patients compared with control subjects when the lecithin/sphingomyelin ratio was ≥ 2 .⁵ The lower phosphatidylglycerol/phosphatidylinositol ratios were observed in patients who were delivered of the relatively heavier babies and who had a much higher incidence of hypoglycemia, features suggesting poor metabolic control in the mothers.⁵ It has been proposed that in poorly controlled diabetes fetal myoinositol levels will not decrease as expected in normal pregnancy as term approaches. The excess myoinositol levels will result in the continued synthesis of phosphatidylinositol instead of the normal conversion to phosphatidylglycerol synthesis.¹⁶

The fact that there were some deviations from the normal regression line, especially in Classes C and D, F, and R (Figs. 2 and 3), although statistically insignificant, is worrisome. The number of patients studied in these classes was relatively few compared with the number in Classes A and B and the number of control subjects. Any subtle effects that the more advanced cases of diabetes may have, might have been diluted and became inapparent. Further studies of these classes in particular are necessary. Meanwhile, while the results of this study suggest that lecithin production is not affected in well-controlled diabetes, we believe it is necessary to continue the practice of documenting fetal lung maturity before elective timing of delivery in diabetic patients. We caution against the delivery of diabetic patients at 38 weeks without documenting fetal

lung maturity, even in well-controlled diabetes, as has been proposed recently.¹¹

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A complete list of references will be furnished on request.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. KENNETH R. KELLNER, Gainesville, Florida (Official Guest). Sometimes complex problems can be reduced to simple concepts. Such is the case with many obstetric situations, which can be reduced to the simple

question, "Is the baby better off in or out?" Under normal circumstances this is not a major issue as nature dictates the answer and we happily defer to her. However, when maternal disease complicates the pregnancy, finding the answer can become the center of all our activities.

In practice, the answer to the question comes from weighing risks. We weigh the potential for morbidity and/or mortality of the baby remaining in utero with the potential for morbidity and/or mortality of the baby being in the nursery.

To be weighed, the risks must be quantitated. In years past, this was done at the "gut" level. Now, with our increasing ability to evaluate both the intrauterine environment and the baby's potential for extrauterine functioning, we are able to more objectively quantitate many of the risk factors.

Management of the pregnant woman with diabetes mellitus is a prime example of these concepts. The introduction of insulin in the 1920s brought a dramatic decline in perinatal mortality, further decreased by an increasing intensiveness of neonatal care in the 1950s and 1960s. In the 1970s, improvements in obstetric care have lowered perinatal mortality to levels approximating those in the nondiabetic pregnancy. An important feature of every obstetric protocol is what is called "the timing of delivery," that is, an assessment of where the baby should be. The risk of stillbirth is estimated by biophysical testing, such as antepartum heart rate evaluation or biophysical profile, and biochemical testing, such as measurements of maternal estriol excretion. The risks for the baby in the nursery are evaluated by estimation of gestational age both clinically and by ultrasound and by assessment of organ maturity as reflected in amniotic fluid constituents.

The basic question is "Is the baby better off in or out?" To answer that question we need to know the risk to the baby if it is put in the nursery. The greatest risk in the nursery is from prematurity. If the greatest risk to the premature infant is respiratory distress syndrome, then the prediction of respiratory distress syndrome would have major impact. It is not surprising then that the finding in the 1970s that respiratory distress syndrome can be anticipated or excluded in the unborn fetus by measuring surface-active phospholipids in amniotic fluid opened a new era in the management of the high-risk pregnancy.

Unfortunately, no test is 100% accurate. In a compilation of several reports, Kulovich et al.¹ showed that in 2170 infants with mature lecithin/sphingomyelin ratios before delivery almost 2% developed respiratory distress syndrome. Most of these "false mature" values occurred in infants born to mothers with diabetes mellitus. Strategies to reduce these "false mature" values have included setting a higher value for a mature lecithin/sphingomyelin ratio in the pregnancy complicated by diabetes or the addition of another parameter such as phosphatidylglycerol. While these strategies have been clinically successful, they have not addressed a basic question: Is there a problem in lung function

of the fetus of the diabetic mother or only in the test we are using to measure that function? Although the first question is certainly of scientific importance, the second is of more immediate clinical significance for a given patient.

The present authors measured the lecithin phosphorus concentration in amniotic fluid from healthy and diabetic women. They do not say if there were any cases of respiratory distress syndrome nor do they report the level of diabetic control. They found no difference between the regression lines representing the relation between lecithin phosphorus concentration and gestational age for the normal and diabetic pregnancies. They concluded that this indicates the absence of a significant influence of diabetes on fetal lung maturation. This conclusion agrees with that of several studies of other parameters of fetal lung maturity. However, as reviewed by the authors, other studies have shown different findings and reached the opposite conclusion.

What conclusion can the clinician draw from these studies? It would appear that diabetes may under some conditions affect fetal lung maturation. The most attractive hypothesis is that maternal hyperglycemia leads to fetal hyperinsulinemia which interferes with some of the surface-active components of lung surfactant. It would also appear that some measures of fetal pulmonary maturity are affected by the same pathophysiologic features and some are not. How may we apply these findings in clinical practice? If we are to prevent respiratory distress syndrome in infants of diabetic mothers, we must first strive for optimal metabolic control of maternal diabetes and second effect delivery only after lung maturity has been demonstrated by a measure or combination of measures proved reliable for the prediction of respiratory distress syndrome in the infant of the diabetic mother. Only in this way will we be able to confidently answer the question "Is the baby better off in or out?"

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DR. ROBERT KNUPPEL, Tampa, Florida. Dr. Fadel and associates are to be congratulated for their ongoing work in the field of amniotic fluid maturity studies and the biostatistical evaluations of their techniques. This is an important study as one team of investigators has garnered, during a 5-year period, a relatively large number of cases of gestational and insulin-dependent diabetes.

As we are aware, the complications of respiratory distress syndrome remain an important concern for the practicing physician. Indeed this clinical problem continues to foster creative in vitro work, and much of this work remains provocative, controversial, and contradictory. It is obvious that the demand for continued

animal model research in evaluation and analysis of fetal lung development and its association with diabetes mellitus must continue if we are to improve perinatal care.

One need only remember back 7 or 8 years when the blood glucose control of diabetic patients was less physiologic. Most investigators would agree that the "tight" blood glucose control has improved perinatal outcome, particularly by reducing the hyperinsulinism and reactive hypoglycemia found in the neonatal period. Hypoglycemia is often associated with the exacerbation and/or coexistence of respiratory distress syndrome. The clinical situations continue to prevail where elective intervention in a diabetic pregnancy is timed by the indirect indicators of the mature fetal lung. However, the variability in the tests for fetal lung maturation continue to plague our practices. Dr. Fadel and his associates present us with another option in determining fetal lung maturity from amniotic fluid in diabetic pregnancies. I am not sure, however, if this technique is superior to the lecithin/sphingomyelin ratio with or without the presence of phosphatidylglycerol.

I have several questions for Dr. Fadel. The first question I have arises from my concerns regarding volume variations and no internal standard when utilizing their technique. I am quite sure that these concerns have been addressed by the investigators but I would like to hear their explanations for the possibility of abnormal values when there are differences in amniotic fluid volumes. In addition, when you extract lipids with acetone, are you sure there are no impurities to threaten the reliability of your test results? As I reviewed Fig. 2 and the relationship between amniotic fluid lecithin phosphorus concentration and gestational age, Class C diabetes does stand out and one must question the power of the test. How big a difference would have to exist to show a statistical difference within this number of patients? Furthermore, when the authors plotted the amniotic fluid lecithin phosphorus levels, were they using independent samples or multiple samples from the same patient? As Dr. Fadel is aware, this is an important consideration when dealing with the analysis of variance.

DR. FADEL (Closing). None of the neonates who were delivered after a mature lecithin phosphorus value, that is, ≥ 0.1 mg/dl, developed respiratory distress syn-

drome, which confirms our previous reported experience.^{1,2}

I did not make a special attempt to identify patients with hydramnios, but I know that its incidence in our patients is low. There were no differences in amniotic fluid lecithin phosphorus between the diabetic patients with and those without hypertensive disorders.

The question of the effect of variations in the volume of amniotic fluid on the results of amniotic fluid lecithin phosphorus, a concentration measurement, has been raised before. In a recent article, Nelson and Nelson,³ using a theoretical mathematical model, demonstrated a minimal effect (5% to 10%) in cases of chronic polyhydramnios or oligohydramnios. The effect was more marked in those situations where the volume of amniotic fluid increased acutely from 1 to 4 L during a 1-week period and the lecithin was measured at the end of this week. In these situations there may be a dilutional effect of up to 20%. However, a similar situation in which amniocentesis for fetal lung maturity is indicated is rarely encountered in the clinical setting. Acute hydramnios did not develop in any of our patients.

I agree with Dr. Knuppel that the number of patients studied in Class C is relatively few and did allude to this in my comments. We compared the findings in these patients with those of matched control subjects with the same race and sex of the newborn infant and with the same gestational age at the time of amniocentesis. No statistically significant difference between the regression lines was found, neither was there a difference in the incidence of mature lecithin phosphorus at the different gestational weeks. Nevertheless, I believe that more studies of these Class C patients as well as Class D, F, and R patients are still needed to confirm the absence of an effect on fetal lung maturation.

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Urethral axis and sphincteric function

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Position and mobility of the urethral axis are considered factors influencing urethral competence. Specific correlation between the urethral axis and its sphincteric function is lacking. Eighty-four patients with the symptom of stress urinary incontinence and 31 patients with sensory symptomatology but no urinary incontinence underwent clinical and urodynamic evaluation. This included objective assessment of urethral axial positions and mobility with use of a specially designed protractor. Comparative analysis of urethral axial data was done between 70 incontinent women with objective evidence of sphincteric incompetence and 24 continent women without it. The urethral axis at rest, during bearing down, and in its total excursion were found to be not significantly different and distributed similarly between both groups. Assessment of the urethral axis was found to be not predictive of urethral function. (AM J OBSTET GYNECOL 1986;155:554-8.)

Key words: Urinary incontinence, sphincteric function, urethral axis, urethral mobility

It is now accepted that proper interpretation of lower urinary tract symptoms requires correlation with objective diagnostic observations.¹ In evaluating the symptom of stress urinary incontinence (involuntary loss of urine during exertion), the assessment of urethral sphincteric function becomes essential. Position and mobility of the urethral axis are considered factors affecting urethral competence, and several imaging and nonimaging techniques to evaluate it have been described.²⁻⁴ However, specific evaluation of the relationship between the urethral axis and its sphincteric function is lacking.⁵ To provide such information, this study compares objective clinical and urodynamic observations of urethral function with assessment of the urethral axis. It will attempt to determine specifically whether urethral position and movement can predict urethral competence or incompetence.

Material and methods

Eighty-four patients with the symptom of stress urinary incontinence (incontinent group) and 31 patients without the symptom of urinary incontinence (continent group) constituted the clinical material for this study. Subjects in the latter group complained of sensory symptomatology, including urinary urgency, frequency, and/or pelvic pressure, but did not complain of involuntary urine loss. All patients underwent a systematic diagnostic evaluation consisting of history,

physical examination, urine culture, retrograde subtracted provocative cystometry, direct visualization of fluid loss, urethral profilometry, determination of urethral axial position and mobility, uroflowmetry, and urethroscopy. Descent of the anterior vaginal wall was assessed during pelvic examination. It was categorized as $\leq 2^\circ$ when the vagina did not descend farther than the midvaginal plane during bearing down and $> 2^\circ$ if it did. Radiographic evaluations or special cultures were obtained when indicated clinically. The patients included in the study did not demonstrate evidence of urinary tract infection, detrusor or urethral instability, urethral diverticulum, obstructive urethropathy, genitourinary fistula, or ventral bladder hernia.

Objective assessment of urethral sphincter function

Direct visualization test. The bladder was filled with room-temperature sterile saline solution. The rate was determined by gravity flow and was discontinued when the patient experienced the sensation of fullness. Overdistention of the bladder was avoided by not filling it in excess of 500 ml. While in the lithotomy position, the patient was requested to cough forcefully. The test was judged "positive" when fluid was seen exiting the urethral meatus at the peak of the cough effort and negative if it was not.⁶

Dynamic urethral profilometry. The technique used follows that described by Asmussen and Ulmstead⁷ and standardized by Anderson et al⁸ and Hilton and Stanton.⁹ All patients were studied in the lithotomy position with their bladders filled to maximum cystometric capacity. An 8F microtip transducer catheter (Millar Instruments, Houston, Texas) with lumen and two pressure sensors located 6 cm apart was initially introduced in the bladder. The catheter was then mechanically pulled out at the rate of 1 mm/sec with the urethral

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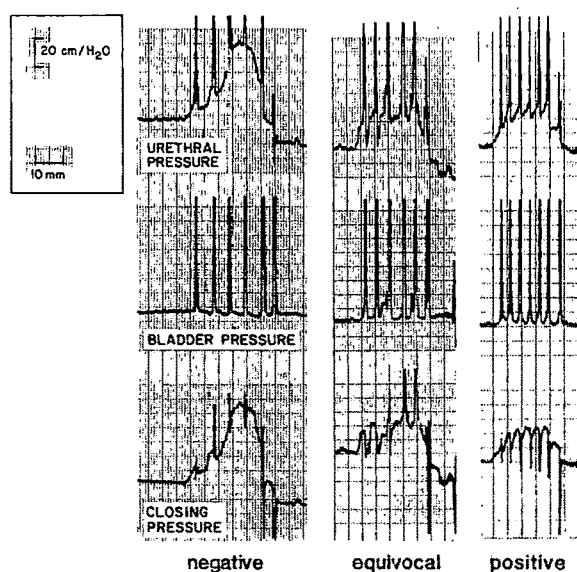


Fig. 1. Dynamic urethral profilometry. *Negative:* The closing pressure does not reach zero during coughs through the functional urethral length. *Equivocal:* The closing pressure reaches zero during some but not all the coughs through the functional urethral length. *Positive:* The closing pressure reaches zero during all coughs through the functional urethral length.

sensor oriented at 9 o'clock. Electronic subtraction of the bladder pressure (distal sensor) from the urethral pressure (proximal sensor) determined the urethral closing pressure. The effect of coughing on the closing pressure was obtained by requesting the patient to cough forcefully while the proximal sensor traversed the urethra. The test was judged positive when zero closing pressure was reached with each cough throughout the functional length of the urethra. It was judged equivocal when zero closing pressure was reached during some but not all of the coughs through the functional length of the urethra. The test was judged negative when the closing pressure during coughing was above zero with every cough through the functional length of the urethra (Fig. 1).

Determination of urethral axial positions and mobility. This evaluation was based on the Q-tip test described by Crystle et al.⁴ The position and movement of the urethral axis was determined with a specially designed protractor mounted over a crenated shaft (Fig. 2). A lightweight metallic Q-tip was placed in the urethra and held against the protractor by a retractable groove. A string level was mounted over the Q-tip, and the protractor was raised or lowered until the horizontal was reached. The groove was retracted and the Q-tip left unsupported. The position at rest was recorded. The patient was then requested to bear down with maximum effort, and the bearing-down position was recorded. The total excursion of the Q-tip was calculated.

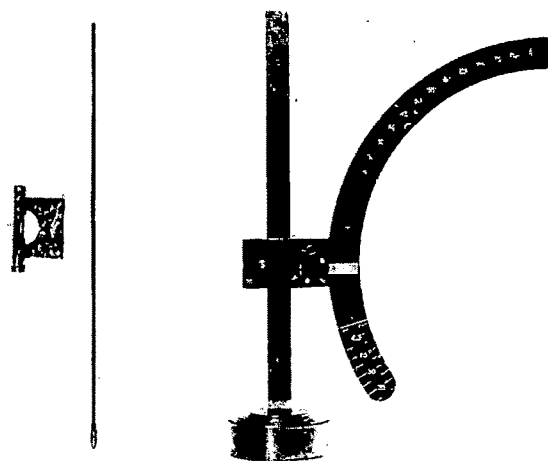


Fig. 2. Determination of urethral axial positions and mobility. See text for description of use of protractor with 5° angle unit measurement, lightweight metallic Q-tip, and string level.

If two consecutive measurements differed, a third one was taken and the average calculated. The protractor's scale has 5° measurement units. Measurements below the horizontal were referred to as negative and above as positive. Comparative analysis of urethral axis data was carried out between incontinent patients with objective evidence of sphincteric incompetence and continent subjects without it. Therefore, excluded from the analysis of urethral axis were incontinent subjects not revealing either a positive direct visualization test or positive dynamic profilometry and continent patients for whom either or both sphincter function tests were positive. These criteria resulted in the selection of the following subgroups: 70 incontinent women who complained of urinary incontinence during exertion and had clinical and/or urodynamic evidence of urethral incompetence and 24 continent women who did not complain of incontinence and displayed no clinical or urodynamic evidence of sphincteric incompetence. Data on their urethral axis position and mobility were analyzed and compared. Except when indicated, definitions and terms conform to those proposed by the Committee on Terminology of the International Continence Society.¹⁰

Results

Characteristics of the subjects incorporated in the study are shown in Table I. There were no significant differences between the two study groups with respect to age, parity, weight, and previous pelvic surgery for reasons other than prolapse and/or urinary incontinence. Differences were noted regarding the degrees of anterior vaginal wall descent and incidence of previous surgery for prolapse and/or incontinence. Seventeen (20%) incontinent subjects had undergone such

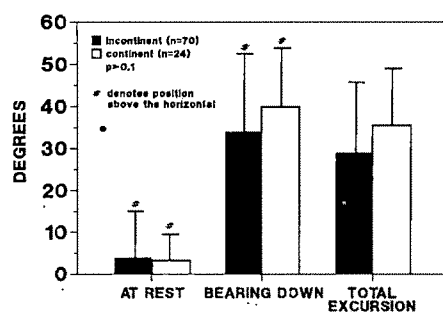


Fig. 3. Mean positions and mobility of the urethral axis in incontinent and continent patients.

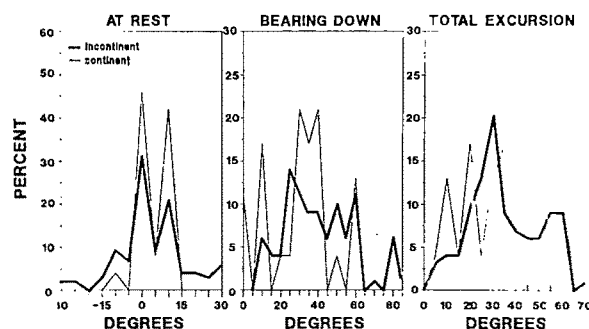


Fig. 4. Distribution of urethral axis data on incontinent and continent patients.

Table I. Characteristics of incontinent and continent patients

	Incontinent group (n = 84)	Continent group (n = 31)	Difference between groups
Age (yr)	45.7 ± 13.6	48.2 ± 12.9	N/S
Parity (n)	2.2 ± 1.5	2.7 ± 1.7	N/S
Height (in)	64.5 ± 3.9	64.3 ± 2.0	N/S
Weight (pounds)	155 ± 35.2	167.7 ± 36.3	N/S
Anterior vaginal wall descent (≤2°)	79 (94%)	23 (74%)	p < 0.01
(>2°)	5 (6%)	8 (26%)	p < 0.01
Previous pelvic surgery other than for prolapse or incontinence (n)	19 (22.6%)	8 (25.8%)	N/S
Previous surgery for prolapse and/ or incontinence (n)	17 (20%)	0 (0%)	p < 0.02

Incontinent patients had symptoms of stress urinary incontinence. Continent patients had sensory symptomatology but no urinary incontinence. Both groups had no other objective lower urinary tract dysfunction.

surgery as compared to none in the continent group. The degree of anterior wall descent was similar in both study groups when those 17 patients were not considered. The observations on the objective assessment of urethral sphincteric function are shown in Table II. Urethral axial positions and total excursion of the selected subgroups are shown in Fig. 3. The mean positions at rest and while bearing down, as well as the total excursion in both study groups, were found not to be statistically different. The percentile distribution of axial data between incontinent and continent subject overlaps (Fig. 4). The characteristics of the two subgroups were similar to those of the original study groups. Data on urethral axis of subjects previously operated for prolapse and/or incontinence were similar to those not undergoing such surgery. Differences in degrees of anterior vaginal wall relaxation did not alter the distribution of data.

Comment

The relationship between urethral position, mobility, and function remains a matter of controversy. Green¹¹ emphasized that the lack of an angle between the urethra and the bladder base was characteristic of stress

incontinence and that posterior rotation of the urethral axis was an indicator of the severity of the condition. Hodgkinson¹² indicated that if the urethral position resulted in the urethrovesical junction becoming the most dependent portion of the bladder base, sphincteric incompetence would occur. McGuire et al.¹³ mention urethral hypermobility demonstrated fluoroscopically as objective evidence of sphincteric failure. Asmussen¹⁴ postulates that it is impaired transmission of pressure from the abdomen to the urethra which induces sphincteric incompetence during exertion. Such condition is said to result from the downward displacement of the urethra below the level of the urogenital diaphragm, and thus relates transmission deficits to urethral movement.

The female urethra derives its closure pressure from all its anatomic components: smooth and striated muscle fibers, mucosa, elastic-connective tissue fibers, and vasculature. During exertion the urethral resistance is challenged. Two protective mechanisms have been recognized: transmission of intra-abdominal pressure to the urethra and a reflex contraction of striated muscle fibers.^{14, 15} Competent or incompetent sphincteric function of the urethra seems, therefore, dependent on various static and dynamic issues. Hilton and Stanton⁹

Table II. Results of direct visualization tests and dynamic urethral profilometry in incontinent and continent patients

Group	Direct visualization test				Dynamic urethral profilometry					
	Positive		Negative		Positive		Equivocal		Negative	
	n	%	n	%	n	%	n	%	n	%
Incontinent (n = 84)	52	62	32	38	50	59	24	29	10	12
Continent (n = 31)	1	3	30	97	0		6	19	25	81

Incontinent patients have symptoms of stress urinary incontinence. Continent patients had sensory symptomatology but no urinary incontinence. Both groups had no other objective lower urinary tract dysfunction.

pointed out that the "margin for continence" was affected by such factors as pressure transmission ratio, amplitude, stability, and sustained response to stress of the maximum urethral closing pressure, as well as variations of intra-abdominal pressure.⁹⁻¹⁶ The role of the urethral axis in relation to these factors is poorly understood and deserves further research. Although it seems reasonable to assume that pressure transmission will be affected by axial position and excursion, data supporting this hypothesis are not yet clear. As urethral competency is the result of several factors, compensatory mechanisms may vary from individual to individual and play a different role in each case. Reflex contraction of striated muscle fibers, for instance, could become the primary mechanism when pressure transmission is impaired and vice versa. Both may become irrelevant in the presence of a very minimal closing pressure at rest, etc.

Most reports concerning urethral position and movement in urinary incontinence have lacked comparative observations on continent controls. The selection of patients incorporated in this study provided the opportunity to compare urethral axial data in subjects with and without symptoms of urinary incontinence. Within these groups, data on urethral positions and mobility were compared specifically between those patients showing objective evidence of either sphincteric incompetence or competence. The urethral axis at rest, during bearing down, and in its total excursion were found not to be significantly different and were distributed similarly between subjects who were sphincter competent and those who were sphincter incompetent. Although urethral position and mobility may influence any one or all of the factors affecting sphincteric competence, the data analyzed here indicate that such anatomic characteristics alone are not predictive of urethral function.

The evaluation of urethral axial position and movement is easy, inexpensive, and reproducible. It provides information about the degree of urethral detachment which direct visualization of the anterior vaginal wall

cannot accurately assess. It has potential use in the selection of operative approach, although this requires further research. The data presented here indicate, however, that it should not be used as a clinical predictor of urethral incompetence or competence.

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Discussion

DR. A. CULLEN RICHARDSON, Atlanta, Georgia. Dr. Fantl, you are in effect saying that axial rotation is not a significant parameter to measure in patients with stress urinary incontinence, which is generally contrary to what we have all thought and taught through the years.

There are several things in the study as you reported it that are not clear. It appears that you only measured the rotation and in turn the downward displacement of the vesical neck. Did you correct for any outward projection of the urethral meatus as is seen when there is a major defect in the pubourethral ligaments?

Most of the references to descent and rotation are drawn from chain-bead studies which place a flexible structure in the urethra. With any rigid structure in

the urethra there inevitably would be some splinting effect if the major descent were in the mild or distal urethra. Was this measured or even estimated?

You compared two groups of patients, but it is not clear how the control group was drawn. Certainly we have all seen patients with marked descent and rotation of the vesical neck who have no stress incontinence—but usually most patients with true stress incontinence do in fact have some descent and rotation of the vesical neck and in turn loss of the posterior urethrovesical angle. Further, if the operative treatment restores the posterior urethrovesical angle, the patient will be generally cured of any true stress urinary incontinence.

If your point is that there is another as yet unidentified factor that must be present in addition to descent of the vesical neck to explain stress urinary incontinence, I would be in full agreement. If you are saying that rotational descent of the vesical neck is of no importance, then I would be in total disagreement; I do not feel that one could draw the latter conclusion from this study.

Extraperitoneal lymph node dissections with use of a midline incision in patients with female genital cancer

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Recent investigations have suggested that extraperitoneal, as compared to transperitoneal, lymph node dissections may result in significantly less morbidity in patients who later have external beam irradiation. Some incisions designed to perform such dissections do not afford easy access to the opposite side. With use of a midline incision carried down to the preperitoneal space, 30 patients underwent bilateral pelvic lymph node dissections. When necessary, access to the para-aortic nodes by an extraperitoneal approach was accomplished by use of a modification of this midline incision. Operating time to complete the pelvic dissection ranged from 35 to 90 minutes. The mean estimated blood loss for the dissection was 135 ml. The advantages of this extraperitoneal approach include easy access to lymph nodes on either side, ability to use the peritoneum as a pack, and an easier access to the obturator space nodes. (AM J OBSTET GYNECOL 1986;155:559-64.)

Key words: Extraperitoneal, node dissection, cancer

The value of a staging laparotomy to evaluate retroperitoneal pelvic and para-aortic nodes in patients with cervical cancer has been noted by several investigators in the past decade.^{1,2} Recently, staging celiotomies have correlated nodal metastases with tumor grade and myometrial invasion in patients with carcinoma of the endometrium.³ Ongoing prospective studies of retroperitoneal nodal metastases in other female genital tract cancers suggest that selected patients should have surgical nodal evaluation when radiographic studies are inconclusive. Serious bowel complications have been noted in cervical cancer patients who have had operative evaluation through a transperitoneal approach followed by radiation therapy.⁴ To avoid these complications, an extraperitoneal approach has been advocated by means of bilateral superior groin incisions,⁵ a unilateral J-shaped incision as described by Berman et al.,⁶ or an upper abdominal incision as described by Schellhas.⁷

With the limitations of these techniques in mind, we have used an extraperitoneal technique that has been successful in staging patients with prostatic or penile carcinoma.⁸ This extraperitoneal approach provides excellent exposure to the nodes without the potential

development of intraperitoneal adhesions, often seen after a transperitoneal lymphadenectomy.

Material and methods

From May 1983 through December 1985, 30 patients with female genital tract malignancies underwent extraperitoneal pelvic lymph node dissection with use of the technique described below. The majority of patients had node dissections for staging purposes. Some patients had pelvic node dissections performed in an extraperitoneal fashion as the initial portion of a radical hysterectomy (Table I). When transperitoneal exploration was not contemplated (that is, surgical staging for advanced cervical carcinoma), a modified midline excision was used to gain extraperitoneal access to para-aortic nodes (Fig. 1).

Operative technique. A vertical midline incision is initially made from just inferior to the umbilicus to the symphysis pubis. The underlying fascia is sharply incised. Care must be taken to carry the fascial incision inferiorly until the symphysis is encountered. At this point the longitudinal recti muscles are bluntly and sharply dissected from the underlying peritoneum. The pyramidalis is sharply sectioned in the bloodless midline raphe, thus affording maximal exposure to the distal nodal areas. The operating surgeon then grasps the superior portion of the peritoneum with one hand, maintaining constant anterior and superior traction. The opposite hand is used to bluntly develop the space of Retzius. A moist spong stick is then used bilaterally to bluntly dissect lateral peritoneum from the iliac vessels and Cooper's ligament. Blunt dissection should continue until the obturator nerve is easily identifiable deep in the pelvis. Often, venous oozing will occur in

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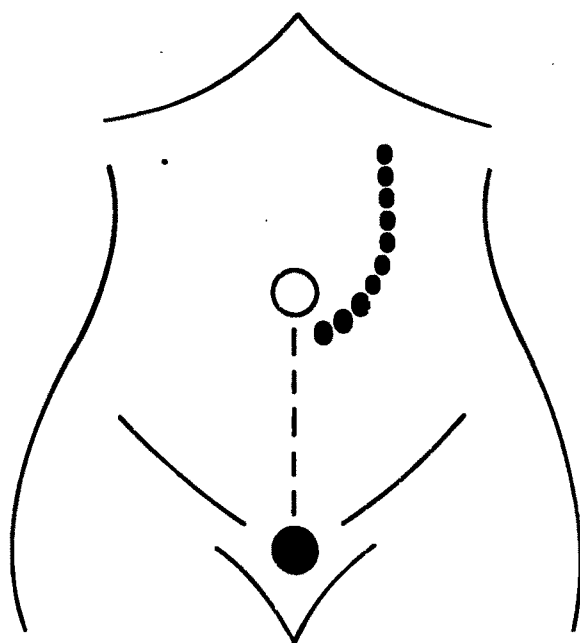


Fig. 1. Extension of midline incision around left of umbilicus for access to para-aortic nodes.

the site of attachment of the peritoneum to Cooper's ligament. This can be readily controlled with cautery. Cooper's ligament should be easily visualized (Fig. 2).

One hand of the operator is then placed anterior to the peritoneum over a lap pad, and the bladder and peritoneum with its underlying contents are pulled away from the opposite pelvic sidewall. Tilting the patient about 15 degrees laterally away from the area of node dissection aids in exposure. A self-retaining retractor can be used. The loose areolar tissue over the external iliac vein is exposed and sharply incised. The fibroareolar and lymphatic tissue is stripped off the vein inferiorly to the limits of distal dissection, the circumflex iliac artery, and vein. Occasionally the inferior epigastric vessels need to be sectioned to facilitate the distal dissection.

The fibroareolar and lymphatic tissue overlying the external iliac artery is similarly mobilized. After identification of the genitofemoral nerve, the lymphatics lateral to the artery are dissected from the underlying psoas muscle (Fig. 3). Vascular clips or a silk tie are placed superiorly at the level of the bifurcation of the iliac vessels. The finger of the surgeon is then passed lateral to the vessels down to the obturator space in order to ensure an avascular plane. With the use of the Singley forceps, all lymphatic tissue is mobilized and passed beneath the artery to the distal limits of the dissection (Fig. 4). At this point, a Kitner dissector is used to strip the remaining areolar tissue overlying Cooper's ligament. Any venous bleeding encountered

Table 1. Extraperitoneal pelvic lymph node dissections

Indications	No. of patients
Surgical staging	
Endometrial carcinoma	5
Ovarian carcinoma*	4
Cervical carcinoma	7
Fallopian tube carcinoma	1
Leiomyosarcoma	1
Mixed mesodermal tumor	1
Preceding radical hysterectomy	11
Total	30

*Two patients with ovarian carcinoma had a pelvic lymph node dissection performed as part of a second-look celiotomy.

can be controlled with cautery. All lymphatic tissue, now in one bundle, can be mobilized medially with the use of a right-angle clamp. A large clamp, such as a Carmalt, is rocked off of Cooper's ligament, and the lymphatic bundle is clamped, sectioned, and tied with silk, flat with the pelvic wall. The iliac vein must be clearly visualized before clamping this pedicle in order to avoid injury.

With the use of the Singley forceps, the lymphatic bundle is bluntly dissected from the obturator nerve and mobilized superiorly. Often the obturator vein and artery must be sacrificed to obtain access to tissue posteriorly and lateral to the nerve (Fig. 5). A Carmalt clamp is placed flat to the pelvic wall, and the bundle is divided and ligated as a single pedicle. Small veins in the obturator space are individually clipped and sectioned. With all lymphatic tissue removed from the space and the lateral bony sidewall clear of tissue, the bundle is mobilized further superiorly. The hypogastric vessels are readily identified. The bifurcation of the common iliac is identified along with the ureter. A Carmalt is used to clamp the lymphatic bundle, sometimes along with the obturator vessels as they course to the hypogastrics. With ligation and division of this pedicle, the lymphatic tissue is removed en bloc. The space is thoroughly irrigated and hemostasis ensured. Two 4 × 8 sponges are inserted into the space, and the opposite pelvic lymphatic tissue is removed in a similar manner. Four sponges, two per side, are left in the area of dissection until completion of any further surgical procedure. Two large-bore closed suction drainage system tubes are eventually inserted into these spaces bilaterally and left in place for 5 to 10 days, depending on the amount of drainage.

In patients selected for an entirely extraperitoneal approach, the skin incision may be extended by a modified midline incision (Fig. 1). Occasionally the left rectus abdominis muscle must be incised to gain adequate access to the para-aortic nodes. Peritoneum is then mo-



Fig. 2. Extraperitoneal space developed by blunt dissection. Cooper's ligament area can be identified. The obturator nerve is seen deep in the pelvis.

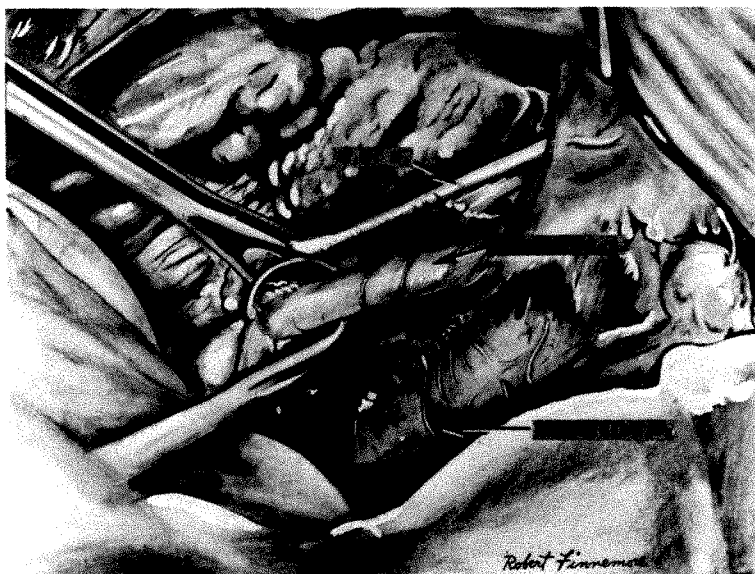


Fig. 3. The genitofemoral nerve (G.F.N.) is cleared of lymphatic tissue. The external iliac artery is being cleansed of its fibroareolar and adherent lymphatic tissue.

bilized medially from the left side. With the use of Deavor retractors the para-aortic spaces are exposed. A para-aortic lymphadenectomy anterior to the great vessels is done. Para-aortic node dissection may be done initially in the staging procedure, once the spaces are developed. Sectioning of the round ligament of the left side sometimes improves exposure in the para-aortic region.

Results

During the study period 30 patients underwent bilateral extraperitoneal pelvic lymph node dissections.

Their ages ranged from 13 to 73; the mean age was 43.3. The 13-year-old had a negative second-look procedure after four courses of combination chemotherapy for endodermal sinus cell tumor of the ovary. The time to complete the extraperitoneal pelvic lymph node dissections ranged from 35 to 90 minutes, with a mean time of 48.7 minutes. The estimated blood loss for extraperitoneal pelvic lymph nodes dissection ranged from 70 ml to 300 ml, with a mean of 135.5 ml. The mean number of pelvic nodes removed was 22.4. Only five of 30 patients (16.6%) had pelvic nodes containing metastatic disease. All five patients had cervical carci-

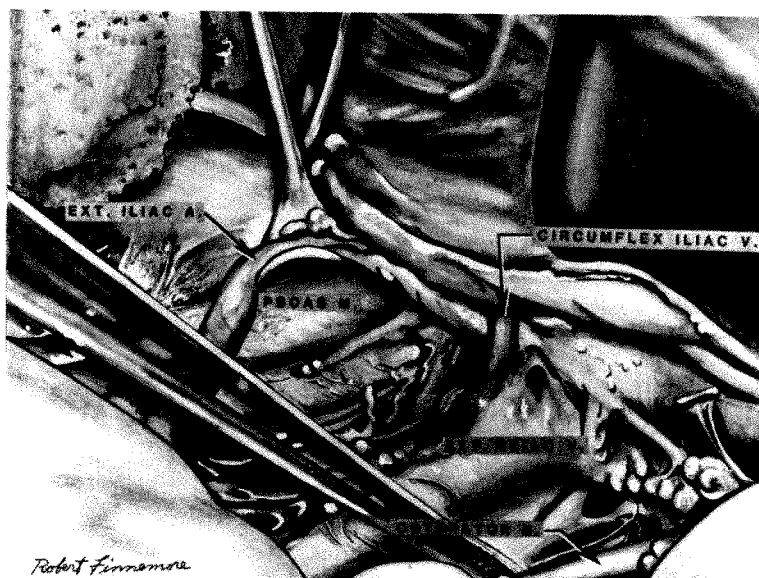


Fig. 4. The external iliac artery is retracted anteriorly as lymphatic tissue is passed medially and posterior to the external iliac vein. The limits of distal dissection, the circumflex iliac vein, crosses anterior to the artery.

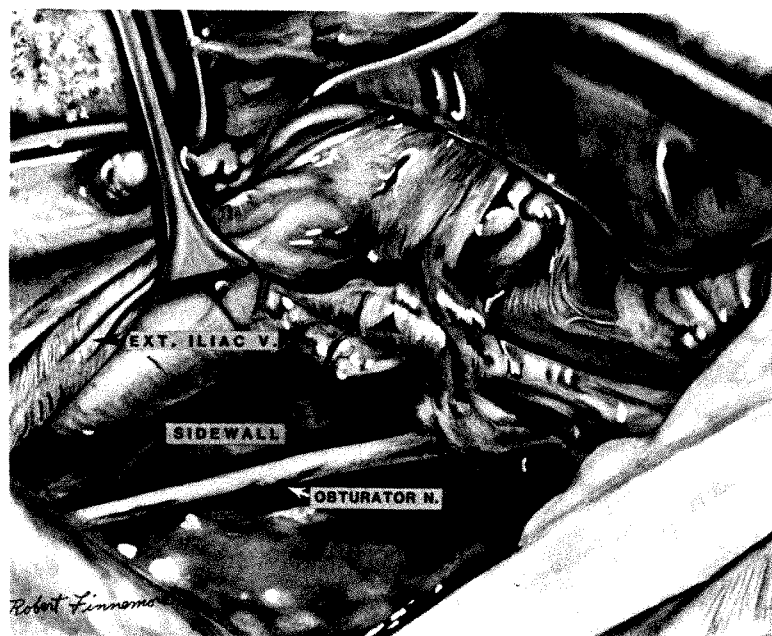


Fig. 5. The arrow points to a silk tie on the most distally ligated lymphatic pedicle adjacent to Cooper's ligament. The obturator nerve is clear of tissue, as is the lateral pelvic wall.

noma. One of these, with Stage IB disease, also had positive para-aortic nodes. Complications were noted in seven patients. Two had lymphocysts; one lymphocyst required drainage. One patient had a superficial wound infection, which was later successfully closed after 5 days of cleansing and debridement. Eight patients required blood transfusions. These patients also had radical hysterectomies performed along with the node dissection procedure.

Comment

An extraperitoneal dissection of pelvic and para-aortic node may decrease the incidence of radiation injury if postoperative irradiation is used. In a series of patients who underwent irradiation because of transperitoneal biopsy-proven para-aortic node metastases, Piver and Barlow⁹ reported that 21.7% died from radiation-induced bowel injuries. Wharton et al.⁴ noted that 27.6% of patients who underwent transperitoneal

node sampling and subsequent extended field irradiation had severe intestinal complications. Fifty percent of those with intestinal complications died. Berman et al.⁶ noted that of 33 patients operated by a transperitoneal approach, 30% required operative intervention for later small bowel obstruction.

An extraperitoneal approach to the pelvic nodes used for urologically related cancers has, in our experience, resulted in easy removal of pelvic nodes. This approach can be particularly valuable in moderately obese patients (18.5% of our patients), because the intact peritoneum can be used as a pack. The technique offers the advantage of a single incision, as compared to a bilateral extraperitoneal approach through separate groin incisions.^{5, 10} The incision can be extended to extraperitoneally excise para-aortic nodes in patients undergoing staging celiotomies for advanced cervical cancer. The para-aortic nodes may be sampled initially once the spaces are developed.

The J-shaped extraperitoneal approach, as described by Berman et al.⁶ has the disadvantage of technical difficulty in excising the right pelvic nodes. The one-incision, pararectal extraperitoneal approach, a transverse incision described by K  lami,¹¹ should also afford excellent exposure for pelvic node dissections. However, access to the para-aortic area would be limited. The single midline incision for extraperitoneal pelvic node lymphadenectomy has been used extensively in patients with prostatic and penile cancer with few complications.^{8, 12, 13} The mean blood loss for this procedure in our experience was only 135.5 ml.

Two patients in the present series developed lymphocysts. Lieskovsky et al.¹³ noted that chronic lymphedema occurred in 41% of their patients. In our opinion, the best method to avoid lymphocysts is to tie the distal pedicle of lymphatic tissue with a permanent suture. Also, retroperitoneal drains should be removed only when the output is 10 to 20 ml in 24 hours. Both patients in the present series had unilateral lymphocysts just superior to the inguinal ligament. Lymphocysts that do not gradually resolve over a few weeks, particularly if they result in severe clinical symptoms, can be managed by repeated aspirations or a marsupialization procedure. A recently reported technique that avoids the risk of infection from repeated aspirations and avoids a major operation is the use of a percutaneous indwelling catheter.¹⁴

In conclusion, the technique herein described offers several advantages. There is an easy access to lymph nodes on both sides of the pelvis and an easier access to the obturator space nodes. The peritoneum can be used as a pack in performing the node dissection, of particular value in obese patients. When a radical hysterectomy is to follow the node dissection, the uterine artery can be easily identified and sectioned at its in-

sertion. Finally, the excellent exposure afforded by this approach allows assisting house officers to view the entire anatomy of the extraperitoneal pelvic space. This may be a useful exposure in selected patients who require bilateral hypogastric artery ligation. The space of Retzius is now developed in the manner described by our residents when performing retropubic urethral suspension.

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Discussion

DISCUSSER 1. It was not clear to me whether any of these patients received postoperative external irradiation. If they did, how many had leg edema permanently?

DISCUSSER 2. How do you expose the iliacs and the paraortic and the vena cava nodes? I was not clear on that from the picture.

DISCUSSER 3. I would worry a little bit about having to irradiate through the midline incision and the pos-

sibility of increasing adhesions close to the incision, as opposed to having an incision off the midline.

DR. GALLUP (Closing). There were three patients in the group that did receive postoperative irradiation. One of the patients who had a lymphocyst did have leg edema. She also had positive pelvic and periaortic nodes, and I think this did play a role in her edema. She subsequently died.

We have not had any problems with the incision. I

must say that our radiation oncologist who I am working with now is not happy about our incisions. But if you look at the J-shaped incision, the incision is still within the radiated field unless you really go out quite laterally, at least in the J-shaped incisions that we have done. But it is a problem. The radiation oncologist wants to wait 2 to 4 weeks, and when we are doing a staging operation, that makes the patient unhappy. It is not an ideal incision from that point of view.

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<i>B. distasonis</i>		
<i>B. ovatus</i>		
<i>B. thetaiotaomicron</i>		
<i>B. vulgatus</i> [‡]		

[†]MEFOXIN is indicated for the treatment of intraabdominal infections, including peritonitis and intraabdominal abscess, caused by *Escherichia coli*, *Klebsiella* species, *Bacteroides* species including the *Bacteroides fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*), and *Clostridium* species.

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**These organisms, formerly considered subspecies of *B. fragilis*, have been given species status; however, because of similarities in their clinical significance and laboratory characteristics, they are often considered together as the "*B. fragilis* group."

[‡]The prescribing information for Cefotan lists no specific indication for infections caused by this organism.¹

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**Conclusion: Cefotan is not
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against the *B. fragilis* group.**

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The evidence **is** conclusive.

fact: At one institution, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus* accounted for 60% of the *B. fragilis* group isolates from 185 intraabdominal infections.²

fact: *B. fragilis* and *B. thetaiotaomicron* are species of great clinical significance and are present in most intraabdominal infections.

fact: Rosenblatt also isolated all five of these *Bacteroides* from clinical specimens at a major teaching institution.³

fact: Chow and Guze reported bacteremia and sepsis due to each of these five members of the *B. fragilis* group.⁴

fact: The prescribing information for Cefotan specifically states that most strains of *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron* are resistant.¹

fact: The absence of susceptibility of these *Bacteroides fragilis* species to Cefotan clearly shows that Cefotan does not have the same anaerobic spectrum as MEFOXIN.

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*i.e., *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron*

††In vitro activity does not necessarily imply in vivo effectiveness.

1. Prescribing information for Cefotan (Stuart), March 1986.

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SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

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Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia: a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: General—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

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Nursing Mothers—Excreted in human milk. Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. **Local Reactions**—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. **Cardiovascular**—Hypotension. **Gastrointestinal**—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. **Blood**—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

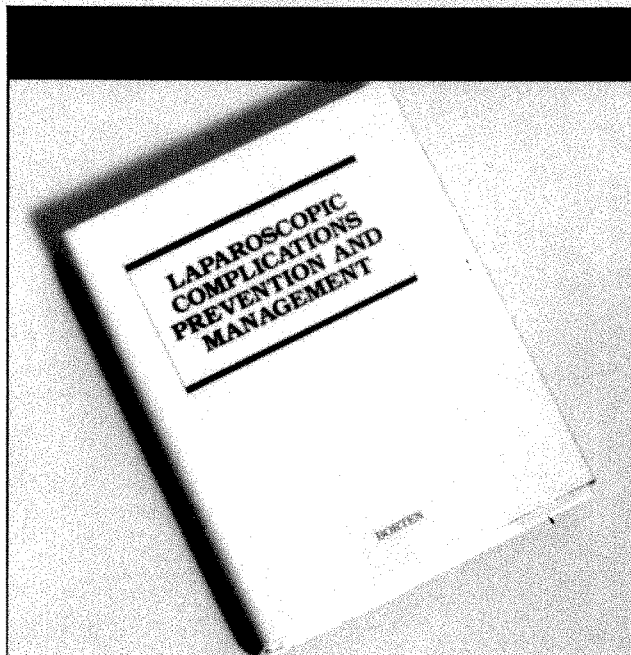
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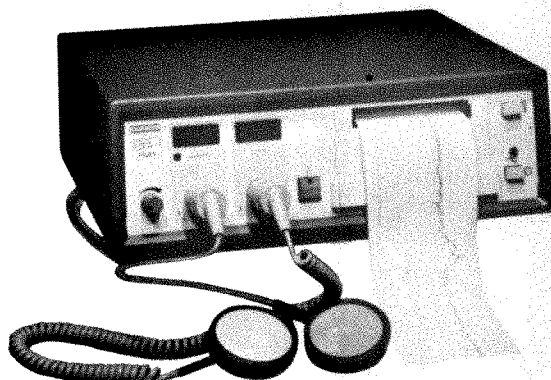
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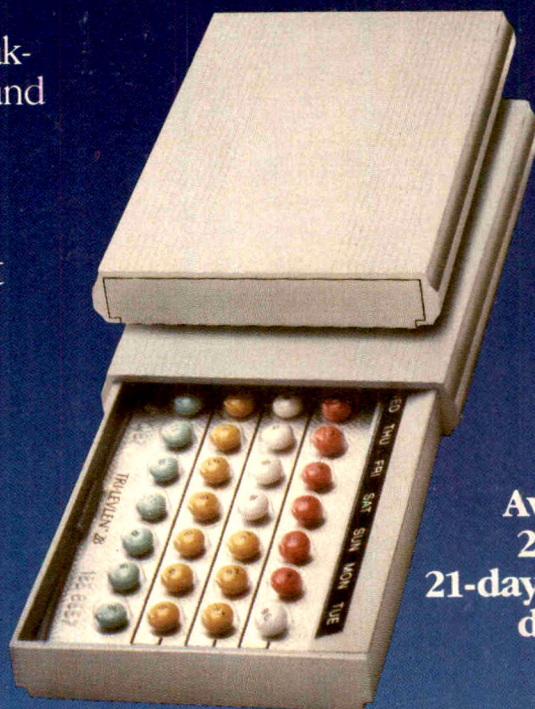
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Indications and Usage.—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications.—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders; 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders; 3. Cerebral-vascular or coronary-artery disease; 4. Known or suspected carcinoma of the breast; 5. Known or suspected estrogen-dependent neoplasia; 6. Undiagnosed abnormal genital bleeding; 7. Known or suspected pregnancy (see Warning No. 5); 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS.—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 5 times greater in users than nonusers.

MYOCARDIAL INFARCTION.—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/torax) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DISEASE.—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES.—A large prospective study carried out in the U.S. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. From an available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preclampsia/torax and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postoperative thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS.—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. Ocular Lesions. There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. Carcinoma. Long-term continuous administration of either natural or synthetic estrogens in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, now currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the 21 cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. Hepatic Tumors. Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that severely and possibly other types of polydactyly are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed

period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. Gallbladder Disease. Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. Carbohydrate and Lipid Metabolic Effects. A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. Elevated Blood Pressure. An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. Headache. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. Bleeding Irregularities. Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in managing breakthrough bleeding, may not be the best approach, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. Ectopic Pregnancy. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. Breast-feeding. OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL.—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalen retention; b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin III; c. Increased norepinephrine-induced platelet aggregability; c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay; Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; d. Decreased pregnaneol excretion; e. Reduced response to metyrapone test.

Information for the Patient.—See Patient Package Labeling.

Drug Interactions.—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis.—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy.—Pregnancy Category K. See "Contraindications" and "Warnings."

Nursing Mothers.—See "Warnings."

Adverse Reactions.—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose.—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration.—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets)—Triphasic Regimen must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

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Clinical Opinion

Drug prescribing for chronic medical disorders during pregnancy: An overview

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Women of reproductive age with chronic medical disorders are often concerned about hazards from drug exposure during pregnancy. The avoidance of any medication after conception may be ideal but is often unwise for maternal well-being. Adverse fetal effects are often not clinically apparent. Current standards for prescribing these medications during pregnancy are discussed from a review of the recent literature. (AM J OBSTET GYNECOL 1986;155:565-9.)

Key words: Drugs, prescription, chronic medical disorders

A challenge for the obstetrician and internist is the proper prescribing of drugs to treat pregnant women with chronic medical disorders. The drug should provide maximal safety to the fetus and therapeutic benefit to the mother. A physician's needs are usually satisfied by a thorough knowledge of one or two drugs in each therapeutic category. Objective and concise information for treating medical disorders during pregnancy may be difficult to find.

Therapeutics is considered in virtually all medical textbooks but often only superficially. Randomized, prospective drug trials in a double-blind manner would be ideal but are often impossible to study during pregnancy. Certain medical disorders occur infrequently at any medical center or cannot be studied during pregnancy because of ethical or regulatory reasons. The purpose of this paper is to summarize current guidelines for prescribing drugs for pregnant women with chronic medical disorders.

Chronic hypertension

The use of home blood pressure monitoring has been especially useful in our practice, since so many women whose blood pressures are elevated in the clinic ($>140/90$ mm Hg) are lower while at home. The most common

antihypertensive drug used during pregnancy is methyldopa, a false neurotransmitter.¹ Maternal sedation, a rare hemolytic anemia, and a positive Coombs test are uncommon but significant maternal adverse effects. Fetal effects are thought to be negligible. A second antihypertensive used during pregnancy is hydralazine. This peripheral vasodilator may cause maternal tachycardia, palpitations, a lupuslike syndrome, and headaches, but no fetal adverse effects are thought to be associated with long-term use.

β -Adrenergic-blocking agents, most notably propranolol, may also serve as second-line drugs for treating chronic hypertension. Although reassuring, minimal information is available about the use of the newer and more selective β -blocking drugs (atenolol, labetalol, oxyprenolol) during pregnancy.¹ Initial reports of a greater incidence of fetal growth retardation, bradycardia, hypoglycemia, and neonatal respiratory distress are likely exaggerated but emphasize a need for fetal surveillance. We try to discontinue the drug 1 day before delivery so that no drug will linger in the newborn infant.

Thiazide diuretics should be avoided to minimize the risk of thrombocytopenia and electrolyte disturbances in both the mother and the fetus. A decreased maternal intravascular volume has been associated with short- and long-term diuretic therapy in pregnant women with chronic hypertension.¹ If diuretic therapy had been given before pregnancy, it may be continued as a single, small, daily dose. It is to be the first drug discontinued if blood pressures become normal during pregnancy. Short-term therapy in large doses should be avoided unless there is pulmonary edema or renal

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failure, since this may diminish uteroplacental perfusion and lead to a greater incidence of fetal hypoxia and death.

The same antihypertensive medications used before pregnancy may be restarted once blood pressures become elevated after delivery. Blood pressure self-monitoring continues to be useful under these circumstances.

Thyroid disease

Drug therapy for hyperthyroidism is the primary form of treatment during pregnancy. The minimal effective dose of an antithyroid drug (preferably propylthiouracil) is recommended although the risk of goiter formation is very low. Propylthiouracil acts by blocking the conversion of thyroxine (T_4) to triiodothyronine (T_3) in peripheral tissues. It is preferred that a hyperthyroid woman requiring propylthiouracil avoid pregnancy until therapy is unnecessary or only when low doses are needed. When propylthiouracil is to be started during pregnancy, the 50 mg tablets are usually prescribed in 100 to 300 mg daily doses with the response being governed by a 3- to 6-week delay before thyroid stores are depleted. Others have reported the successful use of propylthiouracil at higher initial doses (200 to 300 mg twice daily), so that the hyperthyroidism is better controlled in 3 weeks before the medication is tapered.² Serum free T_4 levels should be maintained within an upper normal or slightly elevated range during pregnancy. Methimazole is a satisfactory alternative when rash becomes present in 7% of propylthiouracil-treated persons.

Administering the usual 200 to 300 mg daily dose of propylthiouracil does not apparently pose a hazard to the fetus.² These infants are usually free of clinical evidence of thyroid dysfunction, and T_4 and thyroid-stimulating hormone levels usually revert to normal within the first 5 days. Most physicians are reluctant to encourage breast-feeding for the mother requiring propylthiouracil therapy, despite the findings that only 0.025% of the dose of propylthiouracil taken by the mother is found in breast milk and no changes in the infant's serum T_4 , T_3 , TSH, and T_3 resin uptake levels are anticipated.

Levothyroxine is the preferred drug during pregnancy for women who are hypothyroid. This thyroid supplement contains T_4 alone and appears to be better tolerated than thyroid extract. Thyroid supplements do not pass the placental barrier in appreciable amounts and should not affect the fetal thyroid-pituitary function. The maternal response to thyroid supplementation should be determined by measuring TSH levels. A standard 0.1 to 0.2 mg daily dose (up to 0.4 mg/day) should cause the TSH level to be <10 mU/ml. Serum T_4 levels tend to be high when maintenance

thyroxine therapy is given, which may be explained by differences in potencies between old and new tablets and the time relation after the tablet has been ingested.

Diabetes

Attempts by women with type I diabetes to maintain strict glucose control should begin before conception by daily glucose self-monitoring. Most persons are already accustomed to using the standard beef-pork short-acting (regular) and intermediate-acting (NPH) insulin preparations. Regardless of the metabolic control and duration of diabetes, averaged daily insulin requirements are expected to increase approximately twofold.³ Those times in which insulin doses are anticipated to decrease would be during early gestation because of inadequate caloric intake from nausea and during the third trimester when placental insufficiency is most likely.

Daily self-monitoring of fasting and postprandial glucose values should be done with reflectance glucometers. Insulin is usually administered on two to four occasions each day by late gestation, in a combination of regular and intermediate-acting (preferably NPH) preparations. Experience with continuous subcutaneous insulin infusion therapy during pregnancy has been limited to those with "brittle" diabetes requiring more than three or four daily doses of insulin. Most diabetic women require an average of 1 unit of continuous intravenous regular insulin (range 0 to 2 units) per hour during induction of labor. A low dose of regular insulin may be given intermittently when a cesarean section is anticipated. Insulin demand is expected to drop precipitately after delivery and is rarely necessary for gestational onset diabetes.³ In our experience with type I diabetes, the total insulin dose has been found to be two thirds the prepregnancy insulin dose at the third postpartum day and approximately the same as that before pregnancy by the end of the first postpartum week.

Purified beef-pork insulin preparations and the new biosynthetic, recombinant deoxyribonucleic acid human insulin (Humulin) are becoming no more expensive than standard preparations. These purified products are to be prescribed to women with diabetes, especially those who develop glucose intolerance during pregnancy or have lipoatrophy at prior injection sites. Purification removes potential antigens such as proinsulin and pancreatic hormones and thereby reduces reactions and insulin resistance. No evidence exists that these antibodies or impurities are harmful to the fetus.

Gestational onset diabetic women are eligible for insulin therapy if diet therapy is inadequate. The usual initial dose of 10 to 20 units of Humulin NPH should be started as early in gestation as possible to minimize the chance of the fetus becoming large for gestational

age.⁴ Once insulin is begun, principles for monitoring glucose levels and managing the pregnancy are the same as those for already insulin-dependent women.

Grand mal seizures

We strive to withdraw anticonvulsants before pregnancy after consultation with a neurologist only if the woman has been seizure-free for several years and has a normal electroencephalogram. If she is not pregnant and is taking a combination of anticonvulsants, an attempt should be made to see if the seizures can be controlled with only one agent, preferably phenobarbital.⁵ She should be maintained on as many medications (preferably phenobarbital, phenytoin, carbamazepine) as necessary to control her seizures, since hypoxia from a grand mal seizure is more of a concern.

Anticonvulsants to be avoided because of definite teratogenic concerns include trimethadione (growth delay, mental retardation, facial dysmorphogenesis, microcephaly, developmental delay) and valproic acid (1% open neural tube defects).⁵ Recent information has contested the finding of a fetal phenytoin syndrome (mental retardation, growth delay, microcephaly, facial dysmorphogenesis).⁵ These types of anomalies have been reported in mothers who were either untreated or treated with a different drug. Controversy will likely continue, since this syndrome may represent findings for which any epileptic woman is at risk.

If the patient is first seen after conception and well controlled on her current regimen, the anticonvulsant agents should be continued to avoid precipitating status epilepticus. We advise her that the risk of fetal anomalies is increased twofold to threefold (8% overall) and the changes for mental retardation are increased slightly while she is receiving anticonvulsants.⁵ Except for in utero exposure to valproic acid, routine ultrasonic imaging may not reveal any fetal malformations from the drug exposure.

Anticonvulsant serum levels should be measured at least monthly during pregnancy, with adjustments in dosages being necessary to keep serum levels in a low-normal therapeutic range. Pregnant epileptic women receiving anticonvulsant therapy would be given prophylactic oral folic acid (1 mg daily) throughout gestation to offset any folic acid antagonism from the drugs and oral vitamin K (5 to 10 mg daily) during the last month of pregnancy to prevent a neonatal coagulopathy.

Anticonvulsants should be administered parenterally (phenytoin, 500 mg intravenously during 30 minutes in a non-glucose-containing solution) during labor and delivery to prevent intrapartum or postpartum seizures. Infants should be observed for signs of generalized depression or drug withdrawal. There is no contraindication to breast-feeding for mothers receiving

anticonvulsants as long as the infant shows no signs of generalized depression.

Asthma

Many drugs are available for the control of asthma, and therapy differs little from that of the nonpregnant state. Many patients can be treated solely with intermittent inhalation of β -adrenergic agonists (metaproterenol or albuterol). Two deep inhalations every 4 to 6 hours should be used during short periods of wheezing.

If intermittent inhalation therapy does not suffice, oral methylxanthine therapy should be initiated. The most common of these preparations is aminophylline, and the standard dose is 200 to 300 mg four times daily. Sustained release preparations such as Theodur at a dose of 300 mg every 12 hours may be used for prolonged therapy. The dosage should be adjusted to maintain therapeutic serum levels between 10 and 20 mg/ml. Methylxanthines are thought to inhibit the force but not the frequency of uterine contractions, and their use is associated with an increased risk of prolonged gestation or long labors.⁶ They may also accelerate the development of fetal pulmonary maturity because of the phosphodiesterase-inhibiting property.

Women with confirmed refractory symptoms will require corticosteroid therapy. A single daily oral dose of prednisone (60 to 100 mg) is recommended. Improvement may occur as soon as 6 hours, and the dose is usually tapered over 5 days to 2 weeks. Several studies have failed to show any teratogenic risks, and fetal adrenal suppression is rare.⁶ If prolonged therapy is necessary, a 100 mg intravenous dose of hydrocortisone should be given every 8 hours during labor and immediately post partum.

Migraine headaches

A decrease in the frequency of migraine attacks is often found during pregnancy, especially after the first trimester.⁷ Most reports recommend that ergot alkaloids be avoided during pregnancy because of their theoretical oxytocic effect. This recommendation may be more emotional than scientific, since several preparations are available that contain ergotamine tartrate, which is devoid of oxytocic effects. Ergot alkaloids are relatively contraindicated in the breast-feeding mother because of associated neonatal vomiting, diarrhea, and blood pressure disturbances.

We prescribe simple analgesics such as acetaminophen and codeine as primary drug therapy during pregnancy. Propranolol in daily doses of 40 to 160 mg in four divided doses are often helpful if analgesics are unsuccessful.⁷ Chlorpromazine may be prescribed in a dose of 25 to 50 mg every 6 to 8 hours for acute attacks or 25 mg three times daily for patients with chronic

migraines in addition to propranolol therapy.⁷ Amitriptyline at bedtime may also be used for patients with coexisting depression.

Cardiac disease

Cardiac glycosides during pregnancy are used for treatment of pulmonary congestion unresponsive to rest and diuretic therapy. These agents may also be used for maternal treatment of supraventricular tachycardia, atrial fibrillation, and atrial flutter. Recent case reports of successful therapy of fetal tachycardia have been limited by signs of maternal toxicity. The usual maternal "digitalizing" dose is 0.75 to 1.0 mg intravenously in 0.25 mg increments at 4- to 6-hour intervals or 1.25 to 1.5 mg orally in divided doses. The maintenance dose is 0.25 to 0.5 mg daily.

Monthly determinations of digoxin levels are recommended, since toxicity occurs frequently with any of these preparations. Undesired maternal effects include rhythm disturbances, nausea, vomiting, headache, and neurological imbalances. No adverse fetal effects have been reported, and the concentration in breast milk is low with minimal effects in the infant.

Arrhythmias may occur during pregnancy among women with congenital or ischemic cardiac disorders or myocarditis.⁸ Cardiac glycosides and β -adrenergic blocker (primarily propranolol) drugs are the primary agents. Major advantages to propranolol therapy include the treatment of any coexisting hypertension and of premature atrial or ventricular beats, supraventricular and ventricular tachycardias, and the reduction in ventricular response to atrial fibrillation or flutter or in cardiac output. Originally developed as antianginal agents, calcium channel blockers (verapamil, nifedipine) may also be useful for treating maternal supraventricular tachycardia or inhibiting any premature labor. Cardiovascular side effects in the mother are concerns, and little information is available about the use of these drugs during human pregnancy.

Thromboembolic diseases

The frequency of thrombophlebitis during labor, delivery, and the puerperium has been reported to be fourfold to sixfold that experienced in the nonpregnant state.⁹ The teratogenic effect from warfarin therapy is now unquestioned, and heparin is recommended for anticoagulation during the antepartum and intrapartum periods. An initial bolus of 70 units/kg followed by an infusion of from 300 to 400 units/kg during a 24-hour period has been proposed for initial heparinization. Measuring maternal serum heparin levels, as opposed to partial thromboplastin times, should prevent prescribing high heparin dosages because of the shortening of the partial thromboplastin times by pregnancy-induced alterations. We encourage the heparin

to be regulated to provide a therapeutic level of 0.2 to 0.4 units/ml during the 7 to 10 days of intravenous therapy.

The customary maintenance dose of heparin is 10,000 units subcutaneously twice daily. This may be adjusted to maintain therapeutic serum heparin levels 6 hours after injection. A 5 ml bottle of concentrated heparin (40,000 units/ml) allows for easier self-administration. We continue the heparin therapy with minidoses (5000 units every 12 hours) during labor and omit a single dose when delivery is imminent. Intravenous heparin followed by oral warfarin for 3 months is recommended for women who develop a deep thrombus formation during labor or the puerperium.

Women who have undergone heart valve replacement and are now pregnant are at risk for developing a thromboembolism. Heparin therapy is the preferred anticoagulant, with a subcutaneous dose of 150 to 250 units/kg being given every 12 hours. One or two minidoses may be given during labor. Full heparin therapy may be reinstituted as early as 6 hours after an uncomplicated vaginal delivery.

Persons who have experienced a thromboembolic episode have a 12% risk of recurrence during a subsequent pregnancy.¹⁰ Minidose prophylactic heparin is often recommended. We begin heparin therapy a few weeks before that time of pregnancy when the thrombotic event occurred or by the thirty-fourth gestational week. The therapy is continued post partum until the patient is fully ambulatory.

Streptokinase and urokinase have been developed recently to lyse clots. Because of the significant risk of hemorrhage, these drugs are contraindicated during the antepartum and postpartum periods. A rare exception would be the person at risk for death from pulmonary embolism who did not respond to heparin therapy.

Inflammatory bowel disease

Therapy during pregnancy begins with a modification in diet to avoid lactulose, some fruits, and vegetables. Because there is an increased requirement during pregnancy, calcium intake may need to be supplemented (Os-Cal, one or two tablets three times daily). Codeine or Imodium is not likely to be associated with any increased fetal risk but may be used judiciously during pregnancy only for persistent diarrhea.

Corticosteroids are usually prescribed for pregnant women with ulcerative colitis who fail to respond to the above measures.¹¹ They exert their beneficial effect by accumulating in inflamed bowel tissues and exerting a dose-related inhibition of the inflammatory response. The efficacy of corticosteroids for preventing recurrences in quiescent ulcerative colitis has yielded conflicting results. Corticosteroids may be administered ei-

ther orally or as retention enemas if there is distal colitis. Prednisone in a single daily dose of 40 mg is used initially. A single daily dose is preferable to multiple-dose therapy to minimize adrenal suppression. Most women experience a therapeutic response in 1 to 2 weeks once the disease is better controlled. The dosage is then usually reduced to allow for 4 to 8 weeks of total therapy.

Sulfasalazine is used frequently either alone or in combination with corticosteroids to treat acute ulcerative colitis and acute Crohn's disease during pregnancy.¹² Therapy is likely of little benefit for mild ulcerative colitis only or in the prevention of recurrences. Potential maternal side effects include nausea, anorexia, vomiting, diarrhea, and dizziness. Sulfasalazine inhibits the absorption of folate in the small bowel, so folate supplementation is required. This drug crosses the placenta, and cord blood concentrations have been reported to be approximately half those of simultaneously obtained maternal plasma concentrations.¹² Sulfasalazine is also secreted in breast milk with concentrations approaching 30% those of the simultaneous maternal serum concentrations. No adverse fetal or neonatal effects have been reported with its use, and kernicterus from displacement of bilirubin from albumin is a theoretical concern only.

Azathioprine is an immune antagonist used rarely during pregnancy. Most studies have not demonstrated a significant anti-inflammatory effect during acute inflammation. It may be beneficial for the prevention of recurrences, but there is considerable controversy regarding this issue.

Collagen vascular disorders

Salicylates are the most widely used nonsteroidal, anti-inflammatory drugs for rheumatoid arthritis during pregnancy and may be helpful for the control of minor symptoms in women with systemic lupus. Less is known about newer prostaglandin synthetase inhibitory drugs during pregnancy, but we would assume the same precautions should be considered.¹³ We prescribe enteric coated salicylates in doses of 3.6 to 5 grains daily.

Salicylates cross the human placenta. While there have been occasional reports suggesting an increased risk of fetal malformation, the overwhelming body of evidence has demonstrated no teratogenic effect.¹³ The length of gestation has been reported to be longer, the incidence of postdatism higher, and the duration of labor more prolonged because of the prostaglandin synthetase inhibitory properties. Closure of the ductus arteriosus and pulmonary hypertension in the newborn infant from prostaglandin-inhibiting drugs have not been reported in the fetus but are theoretical concerns. Maternal salicylate ingestion has been associated with

neonatal platelet dysfunction, and an unpredictable increased frequency of intracranial hemorrhage is anticipated for neonates whose mothers ingested the drug.

Systemic corticosteroids are seldom indicated for the treatment of rheumatoid arthritis but form the mainstay of therapy for systemic lupus. These medicines are prescribed in the lowest doses to minimize symptoms and prevent recurrences. A daily dose of 20 to 40 mg of prednisone may be used during exacerbations. If there is major organ involvement (nephritis, central nervous system, vasculitis), the daily dose should be increased to 40 to 80 mg.¹⁴ The only known controlled study of the use of corticosteroids in patients with lupus revealed that those persons who received medication had fewer exacerbations and less perinatal mortality than those who did not receive the drug therapy.

Gold, immunosuppressive medications, and penicillamine have been used to treat selective patients with collagen vascular disorders.¹⁴ Because of a lack of knowledge regarding potential fetal effects, most authorities recommend that these agents be avoided during pregnancy except in life-threatening situations or when more traditional agents have failed.

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A community under siege

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I am not quite sure when the tradition of a presidential address first came to the Association of Professors of Gynecology and Obstetrics, nor am I sure that there is even a file with all of the addresses that have been given. Given that claim to fleeting immortality, I shall try to impart to you those words of wisdom that the title of president implies, with hopefully the same degree of insight as that shown by the Wizard of Oz when he imparted wisdom to the scarecrow, heart to the tin man, and courage to the lion.

I have chosen for the title of my address, "A Community Under Siege," and as customary shall begin by proving to you that first, we are a community, and second, we are under siege.

Of the seven parts of the first definition of the word "community" in *Webster's New Collegiate Dictionary*, academic obstetrics and gynecology fits at least six. We are people with a common interest living in a particular area; an interacting population of various kinds of individuals in a common location; a group of people with a common characteristic or interest living together within a larger society, linked by a common policy; a body of persons having a common history or common social, economic, and political interests; and last, a body of persons of common and especially professional interests scattered through a larger society. Thus, the fact that we are a group with common interests, loosely working together in a common area, with common social and economic bonds certainly enables me to use the term community when I refer to academic obstetrics and gynecology.

In this regard, we are remarkably similar to the Japanese water trade. "Water trade" is the Japanese term used to identify the service industry relating to bars and entertainment. Some of the characters drift in and out, but there is a definite cadre that stays and seems to establish its unwritten, fairly rigid code of conduct. The rewards of catering to your boss or clientele substantial, the punishment for transgression swift. Once

banned, the penance prior to a possible reentry, demanding.

The term siege is most often used to mean a military blockade of a city or fortified place to compel it to surrender, but it may also mean a persistent attack. The usage "lay siege to" perhaps fits our situation best, as the second definition is "to pursue diligently or persistently." We seem to be under persistent attack and definitely pursued persistently. As a smaller community within the larger ones of obstetrics and gynecology and medicine, we have a responsibility to teach at all levels, to replicate ourselves, to do research and expand medical knowledge and improve health care, and to provide direct and indirect services to our patients and colleagues by providing health care and consultation services. While doing all this we must provide for our families, our retirement, and I hope the mental and physical health of our associates and ourselves.

Our teaching responsibilities seem to be going "OK," not excellent, but OK. We still attract about 6% of the United States medical school graduating class. However, last year we were unable to accommodate about 260 United States seniors that expressed a preference for our residencies. We do not know how many others may have selected our specialty but were discouraged by the very tight match and unavailability of first-year residency positions.

We seem to be genuinely concerned with education as an additional skill we must learn. Most of us are reasonably familiar with terminal and enabling objectives, cognitive and noncognitive skills, and normative and mastery examinations. Judging by the response to the education workshops of the Association of Professors of Gynecology and Obstetrics we are willing to put in the effort to acquire education skills so that we can teach, evaluate, and prudently select our future residents. Thus the efforts started in 1968 by the Kennedy Committee of the American Association of Obstetricians and Gynecologists and Dr. Charles Hunter still seem to be paying dividends.

With our limited curriculum time and first-year residency positions we can not afford to be wasteful. Yet, at the other end of the education spectrum, we find a stable failure rate on the written examination of the American Board of Obstetrics and Gynecology and both the board and fellowship program directors are

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concerned regarding the quality of applicants. A significant number of fellows fail part one of the basic board. It is our hope that the new Medical Education Foundation of the Association of Professors of Gynecology and Obstetrics will allow us to find solutions for these and other problems facing our specialty. We should be able to fund faculty interested in medical education, conferences on medical education, and research in medical education techniques.

The major portion of health science research done in this country is carried out in the same academic health science centers that teach and are the single largest source of health care for the indigent. Our departments also provide most of the tertiary referral services and consultations through either our faculty practice plans or supervision of resident services.

Growth income and fringe benefits seem to be slowing. The American Association of Medical Colleges reported our 1984-1985 mean income for full-time faculty members receiving a base and supplement as slightly more than \$94,000. This represented a one-year increase of 10.4% for associate professors, 6.5% for professors, 5.5% for assistant professors, but only 3% for chairmen and less than 1% for instructors. In 1982, when I surveyed the chairmen of obstetrics and gynecology departments regarding fringe benefits, the results were spotty. It was obvious that medical schools were not taking advantage of all the law would allow, and in some cases fringe benefits were sparse. There was in all cases some attempt at a retirement plan and health insurance. Free or low-cost life insurance, tuition benefits for dependents, self-funded retirement plans such as allowed under 403 B, and moving and mortgage reimbursement were often nonexistent or present in limited amounts. Obviously we could do better in terms of planning for our families and our retirement.

What about our mental and physical health? I am ashamed to say that I have no information regarding this aspect of our life. We made one attempt several years ago to discuss impaired or disabled faculty at a chairmen's meeting, but I had the distinct feeling there was little interest. Outside of anecdotal stories, this information seems to be lacking for obstetric and gynecologic faculty. I know of no national effort either to obtain information relating to impaired faculty in our discipline or to aid faculty members that may need it. How many potentially salvageable faculty members drop out of academia because of this? The information is not available.

What about the second half of my title, "Under Siege"? We have four basic functions in a medical school: education, research, patient care, and, as I have painfully learned, administration. Each of these areas seems to have a surplus of problems at the present time.

In the area of undergraduate medical education the

number of applicants to medical school is dropping, and expenses are still rising. The question is, are applications dropping because of lack of interest in medicine as a profession or because of the inability to pay for the cost of a medical education? Medical students pay for their education from a number of sources, but a majority of them have loans. The three federal loan programs are the health professions student loans, health education assistance loans, and guaranteed student loans: The February 15, 1985, issue of the *AMA News* noted that no new federal capital would be allocated to the health professions student loan program and the overall ceiling on health education assistance loans would be reduced from \$250 to \$100 million. Individual guaranteed student loans would be capped at \$4000 and restricted to families with an annual gross income of less than \$32,000. This has subsequently been decreased to a ceiling of \$23,900 for a family of four. In the same issue of the *AMA News*, Sharon Scanlon, a student at Georgetown University School of Medicine, was quoted as noting that this will increase medical students' indebtedness and rule out medical careers for minority and low-income students. The high cost of tuition, coupled with the lack of low-cost loans, the specter of a cap on income, the professional liability situation, and the increasing for-profit sector does not give medicine the aura it once had.

Most of us are already familiar with the painful gyrations that we went through this year while we tried to figure out whether we would have stipends for senior residents and fellows. Now all we have to do is figure out where the replacement dollars are going to come from for those fellows who are funded with patient-generated federal funds. The caps on direct and indirect education pass-throughs, plus the probable lack of Medicaid and Medicare support for fellows' stipends, will, even with ingenious financing, slightly reduce the number of available funded positions. In 1973, Dr. Clyde Randall published the results of a survey of practice patterns of obstetricians and gynecologists. Of those finishing their training in the 10 years before the survey, about 8% were restricting their practice to high-risk obstetrics, gynecologic oncology, and reproductive endocrinology and infertility. If you look at the statistics for last year you will find that about 8% of our residents still enter these fields. It would thus appear that we need slightly under 100 first-year fellowship positions in these three areas. Given the mixture of funding sources for these 100 positions and our lack of dependence on Medicare in obstetrics, I do not think that the change in house staff funding will affect us significantly. The loss of the indirect and direct education pass-throughs will, however, affect us in other areas.

If we look at research funding, we can again feel besieged. The current administration perceives that

Americans are scared of acquired immune deficiency syndrome, adolescent pregnancy, or should I say adolescent sexual behavior, cancer, alcohol, and street drugs. Research funding in other areas will become increasingly harder to obtain as the provisions of the Gramm-Rudman-Hollings act and this administration's need to balance the budget, while at the same time preserving military spending, come into direct conflict. The new investigator will truly have to "walk on water" and also be at a major established research institution, preferably in a state that borders the North Atlantic. A priority score of 152 on a new investigator grant was not funded this year. What will it take next year? In the February 6, 1986, American Association of Medical Colleges president's weekly activities report, Dr. John Cooper notes the demise of the biomedical research support grant program, traditionally the program that gave small grants to new investigators so that they could gather preliminary data necessary to support a National Institutes of Health or National Science Foundation proposal. We can, as chairmen and administrators, make small grants available to our faculty by cross subsidization from our practice income, but that is also being attacked from all sides.

It is difficult to understand the attack on medical education and research funding, but it is truly impossible to understand the attack on health care funding for the poor and elderly. Most of the academic health centers in this country have, since the inception of Medicare and Medicaid, survived and in some cases prospered by collecting charges for patients that were traditionally served free. In many cases this has allowed us to coexist with the private sector without too much friction. As we look at the cap on Medicaid days and reimbursement and the cap on Medicare reimbursement, allowable funds for capital improvements, and support for house staff education, we look longingly at and make efforts to entice full-pay patients into our hospitals in order to support faculty, house staff, and programs that would otherwise have to be abandoned. As we become a greater and greater part of the private sector, we must not abandon our traditional and extremely deserving indigent and near indigent population that has supported us so well.

With this admittedly gloomy picture do I see any hope for funding of indigent care in future? Not under the current administration and with the current philosophy regarding health care.

Perhaps we can come close to understanding, although not agreeing with, the policy if we look at the history of health insurance in the United States. Paul Starr's Pulitzer Prize-winning book, *The Social Transformation of American Medicine* should be required reading for all of us in academic medicine. It is not my

intent to read a book report, but portions are definitely relevant.

"Whoever provides medical care or pays the costs of illness stands to gain the gratitude and good will of the sick and their families. The prospect of these good-will returns to investment in health care creates a powerful motive for governments and other institutions to intervene in the economics of medicine. Political leaders since Bismarck seeking to strengthen the state or to advance their own or their party's interests have used insurance against the costs of sickness as a means of turning benevolence to power."^{*}

Starr's use of Bismarck as the example of a political leader that used health insurance to strengthen the state is appropriate as Bismarck, leader of a totalitarian government, was the first to use health insurance for a healthy work force. Health insurance was not a benevolent fringe benefit but a calculated change to ensure a healthy work force for the state. This is why it expanded to other European countries.

In the United States today, we have a semistable work force with a fairly large component of hard-core unemployable. As we turn from a hands-on, directly labor-intensive manufacturing nation to a work force heavily committed to service industries and automated manufacturing processes that are not labor intense, the component of unemployable among the unemployed will increase. It must seem to someone in the administration that on a cold hard economic basis it does not make sense to provide other than the bare minimum in services for this group to survive—a dismal non-humanitarian thought.

Starr, in another section, continues: "If the first revelations of the seventies had been that a 'health care crisis' existed, the second revelation was that health care hardly affects health. The second revelation obviously made the first revelation seem less important. . . distributive justice is a morally compelling concern, after all, only when what there is to distribute, or redistribute, is genuinely valuable. If it is irrelevant or harmful, the poor would be better off without it."[†]

To continue: "But the most immediate political impact of the new therapeutic nihilism on health policy was to concentrate attention on cost control. If the case for improving access had been weakened, the case for reducing costs was stronger than ever."[‡]

Thus the battle lines are drawn: a government that perceives most health problems to be solved and there-

^{*}From Starr P. *The social transformation of American medicine*. New York: Basic Books, 1982:235.

[†]From Starr P. *The social transformation of American medicine*. New York: Basic Books, 1982:410.

[‡]From Starr P. *The social transformation of American medicine*. New York: Basic Books, 1982:411.

fore little to be served to continuing major research funding except in those areas of immediate public interest, a government that perceives little benefit to be gained from funding health care for the indigent, and a government and a society that view medicine in general as a "fat cat." It is no wonder that we feel besieged.

Obviously it does little to develop this hypothesis unless there is some hope for change. In any siege, either there must be a stalemate, in which the status quo is perpetuated, or one side or the other must surrender or withdraw from the field. I did not use the term victor, as in this case I do not feel there will be one.

The only way that we can survive is to enter the political arena. We must, despite our natural reluctance, support candidates that will support us and let our representatives hear from us often and loud. The Medical Association of Georgia, as have others before, recently had a gathering at the state capital to lobby for tort reform. It was my pleasure to have dinner with one of our legislative delegation the night before that gathering. It just so happened that this gentleman is the cousin of a good friend, and he let me have both barrels. How many physicians knew their legislators? How many had contacted their representatives about issues prior to this? How many physicians had either worked with or financially aided the legislators we now

wanted to influence? He knew the answer just as you and I do, not very many, and he knew as we do that we must do better to survive. We must aggressively push the needs of our patients, our students, and ourselves. If not, no one is going to do it for us. We are relatively few in numbers and we must, when appropriate, establish linkages and coalitions with other groups in order to gain significance in the eyes of those in power.

Despite the lack of funding, the number of abstracts submitted to our national meetings continues to increase. Significant research can take place without National Institutes of Health funding. It is harder, but we can do it. We must let the American public know there is significant research being carried out. They must know that we are conducting research to improve their health, and last, the poor of this country must know that we will not abandon them. They will continue to receive quality health care in the academic health science centers of this country.

In preparing this presidential address I used many sources, but I would like to particularly thank our son Edward for the presents of my holiday reading, *Pictures from the Water Trade*, by John David Morley, and Paul Starr's book, *The Social Transformation of American Medicine*, and also the chance to discuss this address with a true philosopher.

Intrauterine intravascular transfusions for severe red blood cell isoimmunization: Ultrasound-guided percutaneous approach

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Eight pregnancies with severe red blood cell isoimmunization were managed with use of an intravascular approach for intrauterine transfusions. Fetoscopy was not employed, and the procedures were performed percutaneously under direct ultrasound visualization. A total of 16 of 18 attempted transfusions were successfully performed, with four fetuses requiring more than one transfusion. Technical aspects of the procedure as well as its indications, advantages, and drawbacks are discussed. (*AM J OBSTET GYNECOL* 1986;155:574-81.)

Key words: Intrauterine intravascular transfusion, Rh isoimmunization

Intravascular transfusion, both with and without fetoscopic assistance, has recently been reported to be of value in the management of the severe Rh-sensitized pregnancy.¹⁻³ Clinical experience with this approach, however, has been limited. The purpose of this report is to describe eight pregnancies with severe erythroblastosis in which the intravascular route for intrauterine transfusions was used. All transfusions were performed percutaneously under direct ultrasound guidance. The method of transfusion, indications, advantages and drawbacks of the technique employed are discussed.

Material and methods

During a 15-month period from November 1984 to January 1986, 18 intravascular transfusions were attempted at the perinatal ultrasound unit of The Mount Sinai Medical Center. All data were prospectively collected, and no attempted transfusion is excluded from this report.

Method of procedure. After an initial ultrasound assessment of fetal lie, placental location, and umbilical

cord insertion site, the patient is premedicated with a combination of meperidine, prochlorperazine, and diazepam injected slowly by the intravenous route. Cefazolin is also given intravenously for antibiotic prophylaxis.

The technique we use for ultrasound-guided in utero intravascular transfusion is similar to the method described by Jeanty et al.⁴ for amniocentesis. With use of a linear-array real-time transducer, a view of the long axis of the umbilical cord is obtained at its insertion site into the placenta. Holding the transducer in one hand and the transfusion needle in the other, the operator guides the needle tip into the lumen of an umbilical vessel at the placental insertion site (Fig. 1). No special needle guides are used. A 20- or 22-gauge spinal needle is generally used, with the length being determined by the ultrasonically assessed depth from the maternal abdominal surface to the lumen of the target vessel. In situations where the umbilical vein cannot be accessed at the placental insertion site, the needle is directed into the umbilical vein within the fetal liver or into a vessel within the cord at its site of entry into the fetal abdomen. A sample of blood is then withdrawn into a heparinized syringe. Analysis of the red blood cells with an electronic cell sizer is used to confirm that the withdrawn sample is fetal in origin. An initial hematocrit is checked and the fetal blood type is also determined.

Group O Rh-negative packed red cells which have been cross-matched against the mother's blood are then slowly transfused at a rate of 1 to 2 ml/min directly into the umbilical vessel. A final hematocrit is obtained

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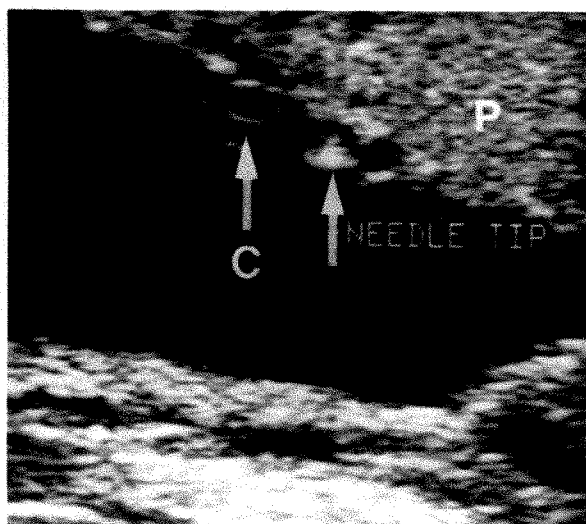


Fig. 1. Sonogram during intravascular transfusion. Arrow points to tip of the transfusion needle in the lumen of umbilical vein. P = Anterior wall placenta, C = cord root.

at the end of the transfusion, the aim being to achieve a posttransfusion hematocrit between 35% and 45%. Fetal heart rate is visually monitored throughout the procedure.

Clinical history, sonographic findings, and procedure-related information for all cases are summarized in Table I, and pregnancy outcome is described in Table II. Case summaries for each patient are described below.

Case reports

Patient 1. S. B., a 29-year-old woman, para 1-0-0-1, group A Rh-negative with anti-D and anti-C titers of 1:256 and 1:32, respectively, at 17 weeks' gestation, was followed with serial amniocenteses. At 31 weeks, the delta optical density at 450 nm (ΔOD_{450}) showed a steep rise into zone III of the Liley curve, the lecithin/sphingomyelin ratio was immature, and there was no sonographic evidence of fetal hydrops. Percutaneous umbilical vein blood sampling revealed a fetal hematocrit of 14%, bilirubin of 6.9 mg/100 ml and blood type of group O Rh-positive. Transfusion of 38 ml packed red blood cells raised the hematocrit to 30%. Fetal pulmonic maturity on amniotic fluid studies was documented 18 days later, and the patient was delivered by cesarean section after a failed induction of labor. The cord blood hematocrit was 23% and bilirubin was 7.4 mg/100 ml. The infant received a total of three exchange transfusions within the first 40 hours of life, and the remainder of the hospital course was uneventful. This case has previously been reported in greater detail.³

Patient 2A. C. M., a 31-year-old woman, para 5-1-0-6, with known Rh isoimmunization, was transferred to our unit at 26.5 weeks' gestation. Ultrasound assessment showed signs of severe fetal hydrops (massive

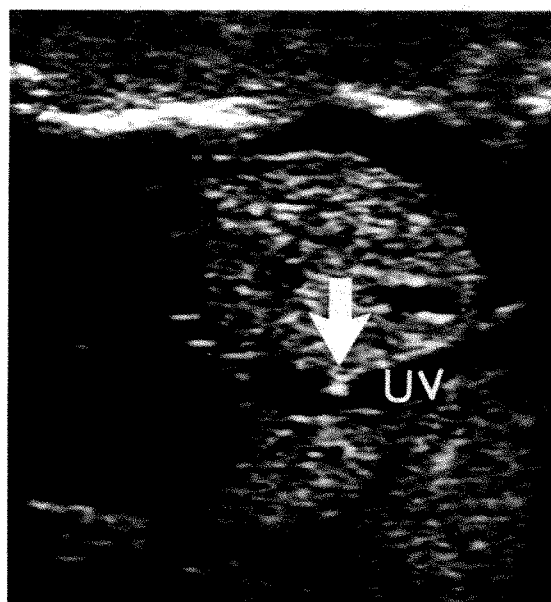


Fig. 2. Sonogram during intravascular transfusion in patient 2A. Arrow points to needle tip in the lumen of intrahepatic part of the umbilical vein (UV).

hepatomegaly, subcutaneous edema, ascites, and pericardial effusion). Severe oligohydramnios was present and no urine was seen in the fetal bladder, although the kidneys appeared to be normal sonographically. It was felt that the oligohydramnios was probably secondary to renal failure resulting from decreased renal perfusion. Intrauterine intravascular transfusion was recommended to expedite delivery of blood into the fetal circulation. The placenta was posteriorly located, and the cord insertion site was obscured by the fetal body. Therefore blood was transfused into the intrahepatic portion of the umbilical vein immediately beyond its entry into the fetal abdomen (Fig. 2). The pretransfusion fetal hematocrit was 2%. Following a transfusion of 45 ml packed red blood cells, the hematocrit was 44%. No evidence of a hematoma within the liver or an increase of free fluid within the abdomen was detected after the needle was removed. Although the fetal heart rate tracing appeared normal for several hours after the procedure, sudden cessation of cardiac activity occurred after 8 hours. Fetal death in utero was confirmed by sonography. The final scan again showed no evidence of bleeding within the liver, and there was no improvement in the oligohydramnios or filling of the fetal bladder. The patient returned to her hometown for delivery. Autopsy on the fetus was refused.

Patient 2B. This patient was seen in her next pregnancy at 13 weeks' gestation with an anti-D antibody titer of 1:2048. Ultrasound assessments of the fetus between 17 and 20 gestational weeks showed no evidence of early hydrops. A percutaneous umbilical blood sampling was electively performed at 21 weeks' gestation, which revealed the fetal blood group to be O Rh-positive, with a strongly positive direct Coombs

Table I. Clinical history, ultrasound findings, and procedure-related information

Patient No.	Previous pregnancies		Blood group and antibody sensitization	Highest antibody titer	ΔOD^{450}		Fetal hydrops
	Parity	Result			Highest level (Liley zone)	Gestational age (wk)	
1	1-0-0-1	Unaffected	A, Rh neg, anti-D, anti-C	1:256 1:32	0.200(III)	31	No
2A	5-1-0-6	Three neonates affected, all needed exchange transfusions	A, Rh neg, anti-D	1:2048	Not done		Ascites, enlarged liver, placental hypertrophy, severe oligohydramnios
2B	5-2-0-6	Same, plus one stillbirth at 26.5 wks	A, Rh neg, anti-D	1:2048	Not done		No No No No No
3	0-1-0-1	Mildly affected; no exchange transfusion	A, Rh neg, anti-D	1:512	0.225(III)	30	Ascites, pleural effusion, cutaneous edema, placental hypertrophy
4	1-0-0-1	Unaffected	AB, Rh pos, anti-Kell	1:128	0.360(III)	29.5	Ascites, enlarged liver, pericardial effusion, placental hypertrophy
5	2-1-3-2	One affected neonate needed exchange transfusions; one fetal hydrops and death in utero at 7 months	O, Rh neg, anti-D	1:8192	0.230(II)	22.5	Ascites, subcutaneous edema, placental hypertrophy Same as above
							Minimal ascites
6	1-0-1-1	One neonate with severe erythroblastosis, required several exchange transfusions	O, Rh neg, anti-D	1:16,000	0.360(III)	22	No No No No
7	1-1-0-0	One stillbirth at 28 weeks, severe erythroblastosis	O, Rh neg, anti-D	1:64	0.300(III)	26	No No No

*Insertion into umbilical vein within the fetal liver.

†Three for intravascular transfusion (IVT) and two for intraperitoneal transfusion (IPT).

‡Intraperitoneal transfusion.

test. The fetal blood had a hematocrit of 22% and bilirubin 5.3 mg/100 ml. A transfusion was immediately performed, followed by four further transfusions at 23, 25.5, 28.2, and 31 weeks (Table I). Elective repeat cesarean section delivery of a 2470 gm fetus was performed at 34.5 weeks after documenting that the lecithin/sphingomyelin ratio was mature. The infant required seven exchange transfusions within the first 48 hours of life, and was discharged home in good condition 17 days after birth.

Patient 3. D. L., a 23-year-old woman, para 0-1-0-1, was referred at 30 weeks' gestation for severe Rh sensitization. The anti-D antibody titer was 1:512, amniotic fluid ΔOD_{450} was 0.225 (zone III of the Liley curve), and the lecithin/sphingomyelin ratio was immature. Fetal scalp edema and ascites were visible on ultrasound examination. The cord insertion was in an anterolateral fold of the placenta. Technical difficulty was experienced during insertion of the needle into an umbilical vessel because of the anatomic location of the cord root

Placental location	Total without transfusion	Gestational age at transfusion (wk)	Total volume of blood transfusion (ml)	Hematocrit		Total needle insertions (m)	Procedure-related complications
				Pre-transfusion (%)	Post-transfusion (%)		
Anterior	1	31.5	38	14	30	1	None
Posterior	1	26.5	45	2	44	1*	None
Anterior	5	21.0	12	22	42	1	None
		23.0	25	17	42	1	None
		25.5	53	12	43	1	None
		28.2	40	23	35	1	None
		31.0	65	22	40	1	None
Lateral	1	30.0	55	12	27	4	Needle tip dislodged and reinserted three times
Posterior	—	29.5	—	11	—	2	Needle dislodged by fetal limb motion two times, suspected cord trauma
Anterior	3	22.5	30	11	35	1	None
		23.5	30	14	45	2	First needle dislodged by contraction; second needle satisfactory
		25.5	60‡	—	—	3†	Technically very difficult for both IVT and IPT
Anterior	4	22.5	20	22	40	1	None
		24.5	30	13	39	1	None
		27.0	45	18	45	1	None
		30.5	50	24	could not be obtained	1	Needle tip dislodged after 50 ml transfusion; final hematocrit not obtained
Anterior	3	26	60‡	—	—	1	None
		28.5	25	35	42	1	None
		31.5	55	19	35	1	None

as well as movement of the fetal legs into the operative area. This continued to be a problem, and it was necessary to reinsert the needle three times during the procedure. The initial hematocrit was 12%, and after 55 ml of packed red cells had been transfused, the hematocrit was 27%. Further attempts at transfusion were discontinued following a final displacement of the needle from the target vessel. Premature labor ensued 8 hours later and was successfully arrested with intravenous magnesium sulfate. However, 36 hours later premature rupture of the membranes and fetal distress necessitated delivery by cesarean section. The infant required a total of four exchange transfusions within

the first 60 hours of life. Associated complications included grade II hyaline membrane disease, which gradually resolved completely. The infant was discharged in good condition after 48 days in the hospital.

Patient 4. K. R., a 24-year-old woman, para 1-0-0-1, was referred at 29 to 30 weeks' gestation with signs of fetal hydrops secondary to Kell isoimmunization. Ultrasound assessment confirmed the presence of moderate fetal ascites and a small pericardial effusion. The amniotic fluid ΔOD_{450} was 0.360 (zone III of the Liley curve), and the lecithin/sphingomyelin ratio was mature. The placenta was located on the posterior uterine

Table II. Pregnancy outcome

Patient no.	Gestational age (wk)	Mode of delivery	Interval between last transfusion and delivery (days)	Weight (gm)	Condition at birth	Cord blood values	
						Hematocrit (%)	Bilirubin (mg/100 ml)
1	34	Cesarean section, failed induction	18	2300	Good	23	7.4
2A	26.5	Cesarean section, repeat	1	—	Stillborn, hydrops	—	—
2B	34.5	Cesarean section, repeat	22	2470	Good	19.0	10.5
3	30	Cesarean section, preterm labor rupture of membranes, and fetal distress	1.5	1550	Hydrops, hyaline membrane disease grade II, VSD, PDA	26.5	7.0
4	29.5	Cesarean section, suspected cord trauma during intrauterine transfusion	0	1470	Hydrops; no evidence of cord or placental hematoma	10.0	2.2
5	25.5	Vaginal	1	840	Stillborn, hydrops	12.0	NA
6	34.5	Cesarean section, repeat	29	2600	Good	21	4.1
7	35	Cesarean section, breech presentation	24	2600	Good	16	4.2

VSD = Ventral septal defect; PDA = patent ductus arteriosus; NA = not available.

*Kleihauer-Betke test.

wall, and its cord insertion site initially could not be visualized. A needle was therefore placed into an umbilical vessel at its entry site into the fetal abdomen. After determining that the hematocrit was 11%, the needle tip became displaced as a result of fetal movement. A second needle was then introduced into the umbilical vein at the cord insertion site into the placenta. This needle, however, also became dislodged because of fetal movement. Further attempts at intrauterine transfusion were then abandoned. Because of concern about possible blood loss secondary to the attempted procedures in a fetus with a hematocrit of 11% and a mature lecithin/sphingomyelin ratio, the patient was delivered by immediate cesarean section. The newborn infant weighed 1470 gm and had a hematocrit of 10% and bilirubin of 2.2 mg/100 ml. The infant required a total of two double-volume exchange transfusions within the first 4 days after birth and was subsequently discharged in good condition.

Patient 5. D. C., a 26-year-old woman, para 2-1-3-2, was referred to our unit at 22.5 weeks' gestation with the diagnosis of fetal hydrops secondary to severe Rh isoimmunization. Her last pregnancy had ended in a stillbirth from fetal hydrops at 7 months' gestation. The anti-D antibody titer was 1:8192, and amniotic fluid ΔOD_{450} was 0.23 (midzone II of the Liley curve). Ul-

trasound assessment confirmed the presence of fetal hydrops with a moderate degree of ascites and scalp edema. Percutaneous umbilical blood sampling revealed the hematocrit to be 11%. A transfusion of 30 ml of packed red cells raised the hematocrit to 35%. One week later, increasing fetal ascites was noted and a second intravascular intrauterine transfusion was performed. This procedure was technically more difficult than the first one, primarily because of a uterine contraction that dislodged the needle tip. This factor, in association with the patient's obesity (220 pounds), necessitated the use of a longer needle, which resulted in successful reinsertion and made it possible to perform the transfusion. The third procedure was scheduled 2 weeks later. Multiple attempts at gaining entry into the umbilical vessels at both the placental and abdominal insertion sites were technically unsuccessful. Therefore an intraperitoneal transfusion of 60 ml packed red cells was administered. Fetal bradycardia occurred intermittently during the intraperitoneal transfusion, although cardiac activity was regular at a rate of 160 bpm at the conclusion of the procedure. No evidence of bleeding from fetal puncture sites or hematoma formation was noted on ultrasound inspection. Eight hours following the procedure, fetal heart sounds could not be obtained by auscultation, and death in utero was

<i>Cord blood values</i>			
<i>Adult transfused red blood cells (%)*</i>	<i>Total exchange transfusions (n)</i>	<i>Neonatal hospital stay (days)</i>	<i>Outcome</i>
50	3	19	Alive and well
—	—	—	Stillborn; autopsy refused
NA	7	17	Alive and well
80	4	48	Alive and well
—	2	40	Alive and well
NA	—	—	Stillborn; autopsy showed hydrops
99	1	18	Alive and well
85	1	17	Alive and well

confirmed by ultrasound examination. The patient returned to her hometown for delivery. The stillborn infant weighed 840 gm, and the cord hematocrit was 12%. Autopsy showed evidence of bilateral pleural effusions, ascites, and hepatosplenomegaly. The placenta was also hydropic and weighed 320 gm. There was no evidence to suggest injury to the cord, placenta, or fetal organs from the transfusion procedures.

Patient 6. C. B., a 26-year-old woman, para 1-0-1-1, group O Rh-negative with history of a previous neonate with severe erythroblastosis, was first seen in our unit at 22.5 weeks' gestation. Anti-D antibody titer was 1:16,000 and amniotic fluid ΔOD_{450} was 0.360 (zone III of the Liley curve). There was no sonographic evidence of fetal hydrops. Percutaneous umbilical blood sampling revealed the fetal blood to be group O Rh-positive with a hematocrit of 22%. The patient received an immediate transfusion and three additional uneventful intrauterine intravascular transfusions at 24.5, 27, and 30.5 weeks of gestation (Table I). Elective repeat cesarean section delivery of a 2600 gm fetus was carried out at 34.5 weeks' gestation after documenting that the lecithin/sphingomyelin ratio was mature. The infant only required a single-exchange transfusion and was discharged home in good condition 18 days after birth.

Patient 7. D. D., a 38-year-old woman, para 1-1-0-0, group O Rh-negative with a prior stillbirth at 28 weeks' gestation secondary to severe Rh isoimmunization, was

referred at 26 weeks' gestation with an anti-D antibody titer of 1:64 and amniotic fluid ΔOD_{450} in Liley zone III. Ultrasound evaluation revealed mild polyhydramnios but no signs of fetal hydrops. An initial intrauterine transfusion was performed at 26 weeks by the standard intraperitoneal approach, followed by two intravascular intrauterine transfusions at 28.5 and 31.5 weeks' gestation (Table I). After documenting that the amniotic fluid lecithin/sphingomyelin ratio was mature at 35 weeks, the patient was delivered by cesarean section for breech presentation. The infant required only one double-volume exchange transfusion shortly after birth and was discharged home in good condition 17 days later.

Comment

Access to the fetal circulation. Until 1981 fetoscopic blood sampling was performed entirely for diagnostic purposes, but with the first intravascular intrauterine transfusion, this route of entry into the fetal circulation was exploited for therapeutic purposes.¹ In 1982 MacKenzie et al.⁵ performed 37 exchange transfusions in utero under direct fetoscopic visualization in fetuses at 15 to 23 weeks before therapeutic abortion. Rodeck et al.⁶ have recently reported their experience with the fetoscopically guided intravascular approach in 25 severely Rh-sensitized fetuses who underwent a total of 77 intrauterine transfusions. Their results were quite impressive, particularly in that subset of patients who received their first transfusion before 25 weeks. In this group 16 of 19 fetuses (84%) survived, including 11 of 13 fetuses who had antenatal evidence of hydrops.

In 1982 Bang et al.⁷ reported the case of a severely erythroblastotic fetus in which they successfully transfused directly into the intrahepatic portion of the umbilical vein at 29 and again at 30 weeks. In these procedures a guide needle was introduced percutaneously under ultrasound guidance into the fetal abdomen 3 to 4 mm from the umbilical vein, and through this a fine needle was advanced into the lumen of the vein. A recent report by Ch de Crespigny et al.² describes the use of this technique to perform a series of 16 transfusions in four Rh-sensitized pregnancies.

In 1983 Daffos et al.⁸ presented a series of 66 cases in which uncontaminated fetal blood was obtained by the percutaneous introduction of a 20-gauge needle directly into the umbilical vein near the placental cord insertion site. These workers have used the same technique to transfuse platelets into a fetus with alloimmune thrombocytopenia.⁹ Our group has previously reported the adaptability of this approach to the performance of intrauterine transfusions in cases of Rh sensitization.³

This series demonstrates that intravascular transfusion was possible in 16 of 18 attempts (89%). In six of our eight cases (75%), a successful outcome was

achieved. In one stillbirth (patient 2A) advanced disease was most likely responsible for the death. In case 5, however, a procedure-related death cannot be excluded. In that case a technically successful intraperitoneal transfusion was administered after a failed attempt to perform the patient's third intravascular transfusion, and death in utero occurred 8 hours later.

Why transfuse intravascularly? In 1963 Sir William Liley¹⁰ performed the first intraperitoneal transfusion for severe Rh sensitization. Since that time this life-saving procedure has been performed on thousands of occasions around the world. Blood placed into the fetal peritoneal cavity can be absorbed via the subdiaphragmatic lymphatics from which it gains access to the circulation. Hydropic fetuses, however, may be too sick to use this mechanism and therefore might derive little or no benefit from a technically successful intraperitoneal transfusion. Furthermore, trauma associated with the introduction of the transfusion needle into the fetal abdomen has in some cases resulted in death in utero. A review of the world literature revealed significant differences in survival rates from one center to another,¹¹ and a recent report of excellent outcome in patients receiving intraperitoneal transfusion notwithstanding,¹² this procedure may not be the ideal form of therapy in all circumstances.

Intravascular transfusion in utero is undoubtedly more technically exacting than the intraperitoneal instillation of blood. Nevertheless, this approach is inherently superior in some respects. By quantification of preprocedure hematocrits and checking of the fetal blood group, inappropriate transfusions can be avoided. Furthermore, when the procedure is indicated, a posttransfusion hematocrit can verify that an optimal amount of blood has been administered. Finally, the direct introduction of blood into the fetal vascular compartment avoids the uncertainty of blood being absorbed from the peritoneal cavity, particularly in hydropic fetuses.

How should intravascular transfusions be performed? The major problem with the fetoscopic approach to intravascular transfusion is that it is technically difficult to perform. Amniotic fluid becomes progressively more opaque as pregnancy progresses, particularly when it becomes stained with bilirubin or hemoglobin pigment. Furthermore, it requires significant skill to puncture a cord vessel under direct vision and then hold the transfusion needle in place while watching it through the fetoscope as packed cells with a hematocrit of >75% are slowly pushed through a 26-gauge needle tip. Rodeck's group has considerable experience with diagnostic fetoscopic blood sampling, but it is not at all clear that other centers will be able to

duplicate their excellent results with this technically demanding procedure.

When comparing percutaneous cord aspiration to fetoscopic blood sampling, Daffos et al.³ enumerate in even greater detail the technical problems associated with the latter technique. Their experience with over 600 diagnostic cases suggests that pregnancy wastage associated with the percutaneous technique is significantly lower than that from even the best fetoscopy series.¹⁴ It is important for other groups to present similar series in order to accurately determine the risk of this procedure, but it should be remembered that the approach is essentially an extension of that currently used in many centers for ultrasonic guidance of a needle into amniotic fluid or parts of the fetal body.

The technique originally described by Bang's group and used in the series reported by Ch de Crespigny and associates involves the introduction of an 18-gauge guide needle into the substance of the fetal liver. The transfusion needle is then passed through this device into a branch of the umbilical vein "at its deepest most fixed point within the liver substance."^{15,7} While neither report describes any evidence of hepatic damage, we were concerned that this could occur if a major vessel was inadvertently lacerated during the procedure. Of course, it could be argued that entry into the cord root at its site of entry into the placenta may result in massive hemorrhage or thrombosis secondary to an associated hematoma. This, however, has not been reported to occur in the large series performed by Daffos et al.¹⁴ or in any of the growing number of cases being done for diagnostic purposes in the United States. Therefore, since entry into the cord root has been more widely used, we feel that it is the preferable approach to be taken whenever possible. It is possible, however, that further experience may show that this method has no significant advantages over the intrahepatic approach.

What are the problems with the percutaneous approach to intravascular transfusion? When performing an intravascular transfusion by the percutaneous approach, the tip of a 20- or 22-gauge needle must be directed into the lumen of a vessel that measures between 4 and 10 mm in diameter. The difficulty of accomplishing this is compounded by the fact that one is trying to enter a three-dimensional space while viewing a two-dimensional image. Nevertheless, with practice, guiding a needle tip into the target vessel is not exceedingly difficult. In our experience, a far greater problem has been inaccessibility of a cord vessel because of intervening fetal parts. This situation requires patience and often manipulation of the fetus to create access to the vessel. Furthermore, when dealing with a posterior placenta, especially in an obese patient, very

long needles may be difficult to guide with precision at depths in excess of 15 cm from the maternal abdominal wall. When this occurs, it might well be more advisable to opt for the intrahepatic approach.

The problem of fetal movement can be significant whether one is working within the liver or with a posterior placenta. Sedation given to the mother is often sufficient to arrest or slow down fetal movement, but this is not always the case. In our series, transfusions in patients 3 and 4 were made more difficult or impossible as a result of physical displacement of the needle from a vessel by fetal limb activity. Ch de Crespigny's group has resorted to the administration of intramuscular curare injected directly into the fetal thigh to eliminate this problem.² They have not been able to detect any adverse sequelae following the administration of this drug, but the reported experience with its use in utero is inadequate to be certain that it is safe.

Finally, the timing of the transfusions and the determination of the optimal amount of blood to be transfused has yet to be established. The latter is particularly relevant in cases of fetal hydrops, in which overtransfusion could conceivably aggravate preexisting cardiac failure. Issues requiring further study include the use of exchange transfusions in utero, and the performance of frequent transfusions of small amounts of blood versus the administration of blood sufficient to normalize the hematocrit with adjunctive diuretic and/or cardiotropic therapy in hydropic fetuses.

In summary, the intravascular transfusion of blood in utero represents a significant step forward in the therapy of severe Rh isoimmunization. Use of ultrasonic guidance for the percutaneous introduction of a needle tip into the umbilical circulation within the cord root or substance of the liver simplifies the performance of these transfusions. Technical problems remain to be solved, but in our opinion the existing approach offers

an alternative to intraperitoneal transfusion with major advantages in selected cases.

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Iatrogenic ureteral injury: Aggressive or conservative treatment

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Many different approaches are advocated for management of iatrogenic (operative) ureteric injury. We herein report our experience with 28 ureteral injuries in 26 patients. In six patients, seven injuries were recognized at the time of the initial operation; four end-to-end anastomoses and three ureteroneocystostomies were successfully performed. In 20 patients the diagnosis of injury was delayed. In 14 of them a ureteroneocystostomy with or without Boari bladder flap was performed. The other six patients were first treated by percutaneous nephrostomy, which was successful in only two cases. The other four needed subsequent operative management. The main point that has emerged from our review is that early definitive operative repair is both feasible and preferable. (*AM J OBSTET GYNECOL* 1986;155:582-4.)

Key words: Iatrogenic ureter trauma, management ureter trauma

Ureteral injuries usually occur during pelvic operations in women. The ureteral damage occurs mainly at the level of the infundibulopelvic ligament at its crossing level with the uterine artery or at its most distal end where it enters into the bladder.

The risk for ureteral damage is increased when the normal course of the ureter is altered by the primary pathologic lesion, but when the ureter is insufficiently identified, every dissection in the neighborhood of the ureter, eventually complicated by bleeding, carries the risk of injury. If the injury is recognized during the operation, a spatulated ureteroureterostomy or a ureteroneocystostomy can be done. Usually, however, the injury only becomes apparent during convalescence.

Material and methods

We have reviewed our experience with 28 ureteral injuries in 26 patients, treated in our department between 1980 and 1984. The age of these patients ranged from 24 to 74 years, and 23 were women; 24 patients showed unilateral and two patients bilateral ureteral injuries.

Table I shows the different types of primary operation during which the ureteral damage occurred. Gynecologic procedures contributed to the majority of ureteral injuries. Ureteral damage occurred during a transabdominal or vaginal hysterectomy in two cases, during an excision of an ovarian tumor in three patients, and during a cesarean delivery in one patient.

Nongynecologic procedures resulting in ureteral injury included aortic operation (two patients, three ureters), sigmoid operation (one patient, two ureters), and appendectomy; stone basket manipulation was the cause of ureteric damage in one patient for a ureteral stone.

Diagnosis was established during the primary operation in six patients (seven ureters) and repaired by ureteroureterostomy (four) or ureteroneocystostomy (three). In the remaining 20 patients ureteral damage became clinically apparent only in the postoperative period. As is noted in Table II, postoperative loin pain, unexplained fever, persistent leukocytoses, and urinary leakage were the predominating symptoms, suggesting the possibility of the ureteral injury. Final diagnosis was established by intravenous urography and retrograde ureteral catheterization. A malfunctioning hydronephrotic kidney and urinary extravasation were the main findings (Table III).

The different types of injuries varying from ligation, partial or complete (13 patients), and transection (10 patients) to excision of a longitudinal segment several centimeters in length are summarized in Table IV. There was one patient with prolapse of the ostium ureteris after stone basket manipulation.

Treatment and results

In six patients, seven injuries were recognized at the time of the initial operation, and ureteroureterostomy or ureteroneocystostomy was performed successfully (Table V). Of the 20 patients with delayed diagnosis, 14 underwent a surgical reconstruction within the first 3 weeks. Treatment delay of >3 weeks was due to uncertain diagnosis in one and the readily apparent, very intense inflammatory reaction in another patient.

In the patient with stone basket manipulation the ureteral injury was recognized only after 2 months and repaired immediately after diagnosis. In one patient a

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Table I. Different types of primary operation

	<i>No. of cases</i>
Gynecologic procedures	
Abdominal hysterectomy	
Total	4
Radical	6
Vaginal hysterectomy	2
Cesarean	1
Excision of ovarian tumor	3
Burch or vaginal colposuspension	2
Total	18
Other procedures	
Vascular surgery	2
Sigmoid surgery	1
Appendectomy	1
Abdominoperineal resection	3
Stone basked manipulation	1
Total	8

ureteral catheter was inserted and surgical repair cancelled because of the severe inflammation at the primary operation. Six patients were treated with percutaneous nephrostomy which was successful in only two cases, the other four requiring a delayed ureteroneocystostomy.

Treatment modalities consisted of ureteroneocystostomy (ureteral implantation according to the Politano-Leadbetter technique) in 18 patients, ureteroureterostomy in four patients, and Boari bladder flap in three patients (Table VI).

Stents were used in all cases requiring anastomosis or ureteroneocystostomy. Of the six patients whose injuries were repaired immediately one patient died in the postoperative period because of extraurinary complications (cardiovascular decompensation). Of the 22 patients who required ureteroneocystostomy or Boari bladder flap, 21 patients had a successful outcome and one patient required additional therapy after 6 months. Percutaneous nephrostomy was successful in only two cases. In the present series, no nephrectomy was performed.

After 1 year the 25 patients were symptom free and intravenous urography results were normal. Intravenous pyelography after 1 year of the patient with the pelvic flap reconstruction indicated a very successful result.

Comment

Perioperative injury of the ureter usually occurs during removal of a large abdominal mass, usually the uterus, extending far laterally. Since actual radiologic imagery such as computerized tomography, permits prediction of operative difficulties, it is recommended that in such cases the ureters be preoperatively catheterized so that perioperative identification is easier.

Table II. Signs and symptoms of patients with surgical injury of the ureter

	<i>No. of patients</i>
Abdominal pain	14
Abdominal tenderness	11
Abdominal mass	2
Fever	8
Anuria	1
Vaginal leakage	3
Wound leakage	4
None	1

Table III. Diagnoses by intravenous pyelography obtained in 21 patients with ureteral surgical injury

	<i>No. of patients</i>
Normal	0
Extravasation	11
Hydronephrosis	18
Nonvisualization	1
Delayed excretion	3
Ureteral narrowing	1

Most unilateral ureteral injuries are recognized 1 or 2 weeks after operation. The most common sign of the compensation is delayed development of a urinary fistula, usually per vaginam but occasionally through the wound. The other signs and symptoms are considered nonspecific. Unusual abdominal or flank pain, distension, fever, or any stormy postoperative course alerts the physician to the complication of intraoperative ureteral injury.¹ In an unknown percentage, ureteral blockage develops without enough signs or symptoms for it to be suspected. Evaluation of unexplained fever, flank discomfort, ileus and abdominal distension, and persistent leukocytosis must certainly include intravenous urography with particular attention to delayed excretion. The exact localization of the injury is best done by cystoscopy and ascending ureterogram. Retrograde pyelography will more accurately depict the precise anatomy in cases of ureteral injury because higher density of contrast material is delivered to the injured site. Temporary percutaneous nephrostomy can be useful in the diagnosis of the site and nature of obstruction or fistula by injecting contrast medium, as for the treatment of small lesions. We had, however, only two successes among the six patients treated this way. Therefore we believe that if the catheter cannot negotiate the damaged ureteral segment, prompt surgical correction is clearly indicated. This is in contrast to the previous belief that preliminary nephrostomy with delayed correction was necessary. By convention the reconstruction and repair of the injured ureter are usually delayed for

Table IV. Type of injury

	No. of ureters
Ligation	13
Partial transfixation	6
Complete ligation	7
Transection	10
Complete	4
Partial	5
Removal of segment	1
Prolapse of the ostium ureteris	1
Necrosis from impaired blood supply	2
Total	28

Table V. Time from primary operation to definitive repair

	Obstruction	Fistula	Totals
Immediate		7	7
1 day	2		2
3 to 7 days	3	5	8
8 to 14 days	1	3	4
15 to 21 days	1		1
>4 weeks	1	5	6
Total			28

several weeks on the grounds that tissue edema and inflammation make reconstruction more hazardous and difficult. This has not been our experience. Reports of definitive repair in the early postoperative period have been published.²⁻⁴ We have found that, within reason, the sooner the reconstruction is attempted, the easier it is to find the tissue planes and restore continuity. The longer the delay, the more difficult the dissection and the more dense the fibrosis. Based on our series of patients, we believe that the advantages of early attack on the injured segment outweigh the disadvantages.

When a ureteric injury is found, reconstruction or reimplantation can proceed without delay, with use of tissues that are fresh and easily identified. The end-to-end reanastomosis is the primary operative procedure of management of the uncomplicated upper and middle ureteral injury.⁵

Regardless of the location of the ureteral injury, if partial transection of the ureter is in question, then formal end-to-end anastomosis with complete transection of the ureter and debridement back to viable tissue is recommended. Simple suturing of partial ureteral transection results in a higher incidence of fistula and stricture formation than ureteroureterostomy. To prevent stricture both ends of the ureter can be spatulated, incised, or obliquely transected so that a wider stoma can be anastomosed. Care should be taken to provide a tension-free anastomosis. This anastomosis can be done over a ureteric stent. The ureteric stent is usually

Table VI. Operative procedure

	Immediate repair	Delayed repair
End-to-end anastomosis	4	
Ureteroneocystostomy	3	6
Ureteroneocystostomy plus psoas hitch		9
Boari bladder flap		3
Boari pyelon flap		1
Percutaneous nephrostomy		6

removed after about 10 days. For injuries to the lower third of the ureter, management becomes a bit more difficult. If the injury is quite low in the pelvis, a ureteroneocystostomy according to the Politano-Leadbetter technique is performed. If there is extensive damage to the lower ureter, then a vesicopsoas hitch or bladder flap procedure may be required. Whatever technique is used, care should be taken to produce a submucosal course of at least 2 cm to prevent reflux. If the ureter is not dilated, a submucosal tunnel of 2 to 3 cm is sufficient; for dilated ureters either a longer tunnel must be performed or a tailoring of the last 5 cm of the ureter is required. The most important point is to ensure that the ureteric reimplantation is done without tension. This is best achieved by suturing the apex of the bladder to the psoas muscle on that side (the psoas hitch).⁶

In the case where lower ureteral injury has occurred and the ureteral defect is too long to perform a ureteroneocystostomy, with or without the psoas hitch maneuver, then construction of a bladder flap will allow sufficient length for anastomosis of the ureter to the bladder. This maneuver facilitates construction of a submucosal ureteral implantation of the ureter into the bladder flap, which will aid in the prevention of vesicoureteral reflux. This procedure has the advantages of the use of well-vascularized bladder wall to bridge the gap to the lower end of the ureter.

Our results with ureteroneocystostomy have been very good, and this procedure is indicated whenever possible.

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Amniotomy and oxytocin treatment of functional dystocia and route of delivery

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The details of clinical management were examined in 101 nulliparous patients with functional dystocia who underwent amniotomy and were treated with oxytocin in the first stage of labor. It was our hypothesis that if the alleged "high" rate of cesarean sections was the result of mediocre or flawed practices, these should be most evident in patients delivered abdominally. A group of 68 patients delivered vaginally are compared with 33 patients delivered by cesarean section. The means of many variables were statistically similar. The cesarean group was characterized by less cervical dilatation at admission, greater birth weights, larger maximum doses of oxytocin, and longer durations of oxytocin therapy. We conclude from our analysis that the decision to perform cesarean section in nulliparous women with functional dystocia arises from disabilities of the patient and not from differences in the application of our management principles, services, or treatments. (AM J OBSTET GYNECOL 1986;155:585-92.)

Key words: Amniotomy, oxytocin, functional dystocia, cesarean section

The current management of functional dystocia in the first stage of labor in patients whose fetuses are in the occipital position consists of rupture of the membranes, if the amniotic sac is intact, followed by continuous oxytocin infusion, if necessary. During the period of evolution of these diagnostic and therapeutic ideas, the rate of performance of cesarean section to effect delivery has risen threefold in the United States but not everywhere.¹ The indication for the largest part of this increase is "failure to progress/cephalopelvic disproportion."² It seems bizarre that reasonably similar diagnostic concepts and therapeutic methods should result in different cesarean section rates unless either the populations or the particulars of management are different. It is the purpose of this work to examine the details of diagnosis and management of functional dystocia in nulliparous women with intact membranes treated by amniotomy and oxytocin. Patients delivered vaginally are compared with those delivered by cesarean section in the first stage of labor. It is our hypothesis that if our "high" rate of cesarean section is the result of mediocre or flawed practices, these should be most evident in the patients delivered by cesarean section.

Methods

Study patients were selected in the following manner. During the approximate year from April 1, 1983, to

March 10, 1984, 300 consecutive cesarean sections were performed in nulliparous women with singleton fetuses. The last nulliparous woman who was delivered vaginally just before each cesarean section and the first who was delivered vaginally just afterward compose the control group (Fig. 1). Of the patients in spontaneous labor delivered vaginally, 242 underwent an amniotomy and 68 of these were treated with oxytocin in the first stage of labor. Of 50 patients in spontaneous labor treated by amniotomy and eventually delivered by cesarean section, 41 received oxytocin in the first stage of labor, and in 33 of 41 complete dilatation was not achieved. The data from the 33 patients delivered by cesarean section are compared with those from the 68 patients delivered vaginally.

There was an occasional missing variable value but never more than one in each group. The Statistical Package for the Social Sciences was used for data analysis,³ and the statistical methods included χ^2 , two-tailed Student's *t* test, one-way analysis of variance, and multiple regression.

Faculty members and obstetrics and gynecology and family practice residents served as the attending physicians. The decision to use oxytocin was the judgment of senior residents or faculty; the choice of cesarean section was the prerogative of the faculty; interim care was provided by the junior residents. No specific oxytocin regimen was used; any dosage schedule recommended in textbooks or in peer-reviewed journals was acceptable.

Results

At admission, the cesarean section group contained more short-statured patients with a higher incidence of

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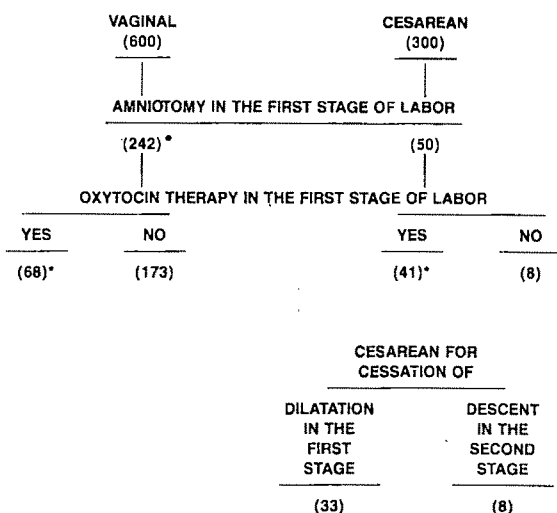


Fig. 1. The plan of the study. Nulliparous patients in spontaneous labor with singleton fetuses (300 consecutive cases formed the cesarean section group). The first nulliparous patient delivered of a singleton fetus vaginally just before and the first delivered immediately after the cesarean section formed the vaginal group ($n = 600$). A total of 2579 nulliparous women who were delivered of singleton fetuses formed the base from which the study patients were selected. *One additional patient received oxytocin in the second stage only; her data are excluded.

Table I. Admission characteristics by route of delivery

	Cesarean section	Vaginal delivery	<i>p</i>
No. of patients	33	68	
Age <20 yr (%)	58	52	NS
Height <60 in (%)	24	9	0.04
Duration of pregnancy			
Preterm (%)	3	8	NS
Postterm (%)	27	26	NS
Admission at first labor room visit (%)	67	59	NS
Admission requested by patient for "labor" (%)	94	94	NS
Pregnancy-induced hypertension diagnosed at admission and treated with magnesium sulfate (%)	48	32	NS

preeclampsia (pregnancy-induced hypertension), who were more likely to be admitted at the first visit to the hospital with the complaint of "labor" (Table I). These differences are not all statistically significant but provide a pattern that could result in earlier admission in labor than is our usual practice and thus account for the fact that the patients undergoing cesarean section had less dilatation at admission (Table II). This difference in the mean cervical dilatation of the two groups dissipated by the time of amniotomy. The cost in elapsed time was great, for the cesarean group spent an average of 5.0 ± 2.1 hours from admission to am-

Table II. Observations from admission to start of oxytocin

	Cesarean section	Vaginal delivery	<i>p</i>
No. of patients	33	68	
Cervical dilatation (cm)			
At admission, mean $\pm$ SD	3.3 ± 1.5	3.8 ± 1.1	0.05
At rupture of membranes, mean $\pm$ SD	4.6 ± 1.7	4.8 ± 1.1	NS
Before oxytocin, mean $\pm$ SD	5.0 ± 2.1	5.3 ± 1.7	NS
Before cesarean section, mean $\pm$ SD	7.1 ± 1.8	—	
Change from admission to rupture of membranes, mean $\pm$ SD	1.1 ± 1.0	1.0 ± 1.0	NS
Elapsed time (hr)			
Admission to rupture of membranes, mean $\pm$ SD	5.0 ± 4.6	2.3 ± 3.0	0.001
Rupture of membranes to oxytocin, mean $\pm$ SD	3.6 ± 3.2	3.1 ± 2.0	NS
Rupture of membranes to oxytocin <2 hr, %	30	33	NS
Rupture of membranes to last examination before oxytocin, mean $\pm$ SD	3.1 ± 3.4	2.6 ± 2.1	NS
Cervical dilatation rate (cm/hr)			
Admission to rupture of membranes, mean $\pm$ SD	0.3 ± 0.4	0.6 ± 0.6	0.01
Admission to rupture of membranes ≥ 1 hr, %	9	19	NS
Last before rupture of membranes, mean $\pm$ SD	0.5 ± 0.9	0.8 ± 0.9	NS
First after rupture of membranes, mean $\pm$ SD	0.3 ± 0.6	0.3 ± 0.8	NS
From rupture of membranes to last examination before oxytocin, mean $\pm$ SD	0.1 ± 0.4	0.2 ± 0.4	NS

niotomy compared with only 2.3 ± 3.0 for the vaginal delivery patients. Twenty-five percent of the patients delivered vaginally and 24% of the cesarean section group showed no cervical dilatation from admission to amniotomy, and there was no difference in the mean change in dilatation of the two groups in that period. Failure to observe dilatation suggests the possibility that such patients may not be in true labor. If this interpretation is valid, the diagnostic error was shared equally by patients delivered vaginally and by cesarean section. The mean cervical dilatation at admission for the vaginal delivery patients who did not have dilatation before amniotomy was 3.8 ± 0.8 cm (range 2.5 to 5.5 cm), and for the cesarean section group who did not have dilatation, 3.7 ± 1.5 cm (range 2.0 to 6.0 cm). The frequency of preeclampsia treated with magnesium sulfate was not significantly different in those patients with dilatation before amniotomy (36%) and those without (44%). There were patients in both groups with dilatation at the rate of ≥ 1 cm/hr before amniotomy but fewer among the cesarean section patients than among those vaginal delivery. If amniotomy had a deleterious

Table III. Additional data

	Cesarean section	Vaginal delivery	<i>p</i>
Birth weight (gm)	3619 ± 507	3378 ± 423	0.02
Elapsed time*			
Admission to delivery, hr	16.0 ± 4.8	10.8 ± 3.9	<0.001
Admission to delivery >12 hr, %	79	23	<0.001
Operative delivery for abnormal fetal heart rate, (%)	3	10	NS
5 min Apgar score <7 (%)	0	1	NS
Dose of oxytocin, mU/min			
Maximum, mean ± SD	6.9 ± 5.9	4.1 ± 3.8	0.01
Maximum <10, %	76	94	0.01
Final, mean ± SD	6.1 ± 6.0	4.1 ± 3.8	0.03
Oxytocin not stopped and dose not reduced (No. of patients)	20 (61%)	54 (79%)	NS
Oxytocin incrementation rate† (No. of patients)			
None	3 (9%)	17 (25%)	
<30 min/mU	14 (42%)	21 (30%)	
≥30 min/mU	16 (48%)	30 (44%)	

*For the cesarean section group, from admission to decision from cesarean section.

†The shortest pair of intervals between dosage changes or the only interval if only one dosage change.

Table IV. Cervical dilatation rates observed at six sequential examinations after start of oxytocin, with data grouped by route of delivery

Sequential examination No.	Patients remaining for study				Elapsed time from last examination before start of oxytocin (hr), average ± SD			Cervical dilatation rate (cm/hr), average ± SD		
	Cesarean section		Vaginal delivery		Cesarean section	Vaginal delivery	<i>p</i>	Cesarean section	Vaginal delivery	<i>p</i>
	No.	%	No.	%						
1	33	100	68	100	1.9 ± 0.7	1.8 ± 1.1	NS	0.2 ± 0.3	0.8 ± 0.9	0.001
2	32	97	62	91	3.2 ± 1.0	3.0 ± 1.2	NS	0.6 ± 0.8	1.5 ± 2.4	0.04
3	30	91	46	68	4.4 ± 1.4	4.1 ± 1.4	NS	0.6 ± 1.5	1.2 ± 1.3	0.07
4	27	82	35	51	5.7 ± 2.1	5.4 ± 1.8	NS	0.4 ± 0.7	1.5 ± 2.2	0.02
5	25	76	23	34	6.9 ± 2.6	6.8 ± 2.0	NS	0.4 ± 0.8	1.1 ± 1.6	0.05
6	19	58	17	25	8.0 ± 2.4	8.0 ± 2.0	NS	0.3 ± 0.5	1.0 ± 1.0	0.01

effect on labor progress, it affected more patients in the vaginal delivery group (19%) than in the cesarean section group (9%). The elapsed time from amniotomy to the start of oxytocin was similar in the two groups, and in each group about one third of the patients were observed for <2 hours before the initiation of oxytocin.

The two groups of patients were different in several respects: birth weight, elapsed time from admission to delivery, maximum dose of oxytocin, and the frequency with which the dose of oxytocin was stopped or reduced (Table III). Only one cesarean section patient had the diagnosis of "fetal distress" in addition to functional dystocia, but seven patients had low-forceps deliveries for "fetal distress." The only patient with a low 5-minute Apgar score was admitted with an absent fetal heart rate and apparent fetal death before labor. Of the patients who received more oxytocin than the initial dose

of 1mU/min, there was no difference in the incrementation rate of the two groups.

The rates of cervical dilatation were computed for the first six examinations after the initiation of oxytocin (Table IV). There is no selection in respect to the frequency with which the examinations were performed, as evidenced by the similarity in the elapsed times to each examination. The groups are different in that the vaginal delivery patients became completely dilated faster than the decision to perform abdominal delivery is made in those with cesarean section. For instance, at 6.8 hours, two thirds of the vaginal delivery group were fully dilated, and at 8.0, three fourths. In contrast, only one fourth of the patients destined for cesarean section had the decision made by 6.9 hours and less than one half by 8.0 hours. The vaginal delivery group of patients had dilatation at a greater rate than did the ce-

Table V. Effect of elapsed time on capacity of rates obtained from first five sequential examinations after start of oxytocin to identify route of delivery*

Sequential examination No.	Total No. of patients	Incidence of cesarean section (%)	Sensitivity (%)	Specificity (%)	Predictive value of abnormal rate (%)
1	101	32	97	38	43
2	94	34	72	38	37
3	76	39	83	51	52
4	62	43	89	53	53
5	48	47	84	43	57

*For evaluation of effect of examination sequence, an "abnormal dilatation rate" is defined as <1 cm/hr.

Table VI. Effect of using standard of two sequential dilatation rates <1.0 cm/hr as definition of "abnormal rate" to identify route of delivery

Sequential examination No.	Total No. of patients	"Abnormal rates"		Incidence of cesarean section (%)	Sensitivity (%)	Specificity (%)	Predictive value of abnormal rate (%)
		No.	%				
2	94	48	51	34	69	78	46
3	76	37	49	39	60	59	49
4	62	31	50	44	70	66	61
5	48	26	54	57	80	75	77

Table VII. Elapsed times from last examination before oxytocin to complete dilatation or decision for cesarean section and from start of oxytocin to final first-stage dose of oxytocin, and their difference, segmented by both cervical dilatation before oxytocin and route of delivery

Cervical dilatation just before oxytocin (cm)	Route of delivery	No. of patients	Maximum dose of oxytocin (mU/min), mean $\pm$ SD	Elapsed time* to complete dilatation or decision for cesarean section (hr), mean $\pm$ SD	Elapsed time† from start of oxytocin to final dose (hr), mean $\pm$ SD	Elapsed time minus duration to final dose (hr), mean $\pm$ SD
All	Cesarean section	33	6.9 $\pm$ 5.9	9.5 $\pm$ 4.8	6.2 $\pm$ 4.7	3.2 $\pm$ 3.0
<4.0	Cesarean section	7	9.1 $\pm$ 9.9	13.8 $\pm$ 2.7‡	9.0 $\pm$ 5.4	4.8 $\pm$ 4.9
4.0 - 4.5	Cesarean section	6	6.7 $\pm$ 3.4	10.0 $\pm$ 4.5	7.3 $\pm$ 5.1	2.7 $\pm$ 1.5
5.0 - 5.5	Cesarean section	9	5.0 $\pm$ 2.4	6.6 $\pm$ 3.8§	4.5 $\pm$ 3.4	2.1 $\pm$ 1.6
6.0 - 6.5	Cesarean section	4	7.8 $\pm$ 6.4	9.4 $\pm$ 2.8	6.4 $\pm$ 4.3	3.0 $\pm$ 1.7
7.0 - 9.5	Cesarean section	7	6.7 $\pm$ 5.9	8.4 $\pm$ 6.4	4.7 $\pm$ 5.1	3.7 $\pm$ 3.6
p Value			NS	0.04	NS	NS
All	Vaginal delivery	68	4.1 $\pm$ 3.8	5.2 $\pm$ 3.5	2.7 $\pm$ 3.3	2.6 $\pm$ 1.9
<4.0	Vaginal delivery	10	5.4 $\pm$ 4.7	9.1 $\pm$ 5.3‡	4.9 $\pm$ 5.3‡	4.3 $\pm$ 3.1‡
4.0 - 4.5	Vaginal delivery	14	4.0 $\pm$ 2.4	4.7 $\pm$ 2.0	2.2 $\pm$ 1.7	2.5 $\pm$ 1.5
5.0 - 5.5	Vaginal delivery	19	3.4 $\pm$ 2.1	5.0 $\pm$ 3.0	2.3 $\pm$ 2.4	2.7 $\pm$ 1.7
6.0 - 6.5	Vaginal delivery	13	5.9 $\pm$ 6.3	5.3 $\pm$ 3.5	3.5 $\pm$ 3.3	1.7 $\pm$ 1.3
7.0 - 9.5	Vaginal delivery	12	2.4 $\pm$ 2.3	2.8 $\pm$ 1.6	0.8 $\pm$ 1.1§	2.0 $\pm$ 1.2
p Value			NS	0.0007	0.02	0.01

*From last examination before the start of oxytocin.

†Elapsed time from last examination before oxytocin to complete dilatation for vaginal delivery patients or decision for cesarean section minus duration from start of oxytocin to final dose change.

‡Student-Newman-Keuls test indicates difference from others at 0.05 level.

§Student-Newman-Keuls test indicates difference from others at 0.05 level.

||The F test probability.

sarean section patients, but the standard deviations of the means are as large as or greater than the mean, demonstrating that at each successive examination each group of vaginal delivery patients contained some patients with very rapid dilatation and others without.

The rate of cervical dilatation as determined prospectively in the realities of clinical experience is an inefficient "test" for predicting the route of delivery (Table V). While the test has modest sensitivity, about 85%, it has low specificity, approximately 45%, and low

Table VIII. Elapsed times from last examination before oxytocin to complete dilatation or decision for cesarean section and from start of oxytocin to final first-stage dose of oxytocin, and their difference, segmented by both approximate quartiles of maximum dose of oxytocin and route of delivery

Maximum dose of oxytocin (mU/min)	Route of delivery	No. of patients	Cervical dilatation just before oxytocin (cm), mean $\pm$ SD	Elapsed time* to complete dilatation or decision for cesarean section (hr), mean $\pm$ SD	Elapsed time from start of oxytocin to final dose (hr), mean $\pm$ SD	Elapsed time† minus duration to final dose (hr), mean $\pm$ SD
All	Cesarean section	33	5.1 $\pm$ 1.8	9.4 $\pm$ 4.8	6.3 $\pm$ 4.7	3.2 $\pm$ 3.1
1-2	Cesarean section	9	5.3 $\pm$ 2.1	6.1 $\pm$ 3.9	2.4 $\pm$ 2.9‡	3.7 $\pm$ 4.3
3-5	Cesarean section	9	5.1 $\pm$ 1.5	8.2 $\pm$ 4.0	4.1 $\pm$ 3.3‡	3.8 $\pm$ 2.3
6-9	Cesarean section	7	4.6 $\pm$ 1.5	10.1 $\pm$ 4.3	8.3 $\pm$ 3.5§	1.8 $\pm$ 1.8
≥ 10	Cesarean section	8	5.2 $\pm$ 2.0	14.3 $\pm$ 3.5‡	10.9 $\pm$ 3.1§	3.4 $\pm$ 3.1
p Value			NS	0.0014	<0.0001	NS
All	Vaginal delivery	68	5.4 $\pm$ 1.7	5.2 $\pm$ 3.6	2.6 $\pm$ 3.1	2.6 $\pm$ 1.9
1	Vaginal delivery	15	6.5 $\pm$ 1.7*	3.1 $\pm$ 2.3	0.2 $\pm$ 0.9‡	2.9 $\pm$ 1.8
2	Vaginal delivery	15	5.3 $\pm$ 1.8	4.1 $\pm$ 2.5	1.2 $\pm$ 1.0‡§	3.0 $\pm$ 2.4
3-4	Vaginal delivery	15	4.5 $\pm$ 1.3	5.0 $\pm$ 2.5	2.9 $\pm$ 2.5§	2.2 $\pm$ 1.8
≥ 5	Vaginal delivery	23	5.2 $\pm$ 1.7	7.4 $\pm$ 4.3‡	5.0 $\pm$ 3.6¶	2.5 $\pm$ 1.8
p Value			0.01	0.0007	<0.0001	NS

*From last examination before start of oxytocin.

†Elapsed time from last examination before oxytocin to either complete dilatation for vaginal delivery patients or the decision for cesarean section minus the duration from start of oxytocin to final dose.

‡Student-Newman-Keuls test indicates difference from others at 0.05 level of probability.

§Student-Newman-Keuls test indicates difference from others at 0.05 level of probability.

||The F test probability.

¶Student-Newman-Keuls test indicates difference from others at 0.05 level of probability.

predictive value of an abnormal result, 50%. The predictive value of an abnormal rate rises if the oxytocin dose exceeds 9 mU/min, in the later examinations such as the fourth and fifth rather than the first or second, and in the patients who experienced stopping of the oxytocin or reduction of the dose because of abnormal fetal heart rates or hypercontractility. The enhanced efficiency of the "test" is entirely the result of the progressively increasing incidence of patients destined for cesarean section with the passage of time because patients become fully dilated at a faster rate than the decision for cesarean section is made. Defining an abnormal rate as <1.0 cm/hr regardless of cervical dilatation or using a standard of <0.6 cm/hr for patients ≤ 4.0 cm dilated and <1.2 cm/hr if >4.0 cm made no difference at all in the effectiveness of rate values in predicting route of delivery.

In a further attempt to improve the predictive power of dilatation rates as a test, the frequency with which two consecutive examinations provided rate values of <1.0 cm/hr was examined (Table VI). The percentage of abnormal tests, sensitivity, and specificity showed little change from the second to fifth examinations, but the predictive value of an abnormal test increased as the incidence of patients destined to be delivered by cesarean section rose. Using two consecutive unsatisfactory rates to predict the route of delivery is not better than using one.

The elapsed time from the decision to use oxytocin

to the election of cesarean section or to complete dilatation is composed of two segments: (1) the time from the last examination before oxytocin to the last dose change and (2) the time from the last dose change to either decision for cesarean section or to complete dilatation. The former represents the time required to identify the appropriate dose, and the latter, the period necessary to observe the cervical dilatation effected by strong contractions. Common wisdom would suggest that the duration of the oxytocin dose identification period would be dependent on the maximum dose required, and the period of observation that follows would be related to the extent of cervical dilatation at the start of oxytocin therapy. Significantly more total time was required by the cesarean section group (9.5 ± 4.8 versus 5.2 ± 3.5 hours, $p < 0.01$), and that group expended a larger share in the "dose-finding" period (6.2 ± 2.7 versus 2.7 ± 3.3 hours, $p < 0.01$) (Table VII). There is no significant difference in the "cervical dilatation observation" period that follows (3.2 ± 3.0 versus 2.6 ± 1.9 hours, $p = \text{NS}$).

Segmenting the data by cervical dilatation before the start of oxytocin demonstrates that both the cesarean section and vaginal delivery groups require significantly longer elapsed times if the dilatation is <4.0 cm (Table VII). For the vaginal group this significant difference for the patients dilated <4.0 cm also pertains to the periods of both dose finding and observation. This result demonstrates that more time is used for patients

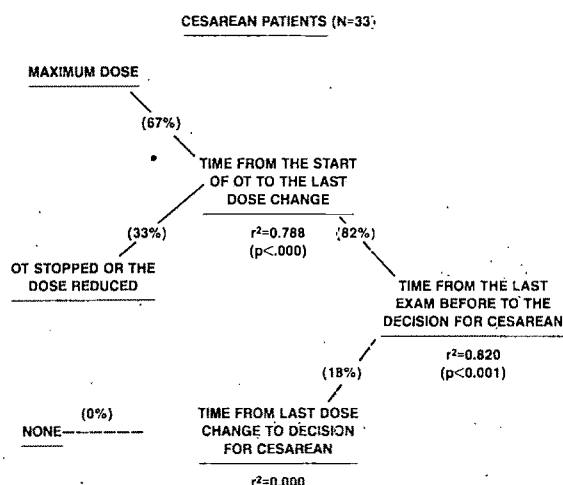


Fig. 2. Multiple regression analysis of the variance in: (1) elapsed time from last examination before start of oxytocin to decision for cesarean section, (2) elapsed time from initiation of oxytocin (OT) to last dose change in first stage, and (3) difference between them. r^2 is the portion of the variance that is explained, and the percent value is the quantity of r^2 accounted for by that variable. For example, 82% of the variation in values for the duration of time before the start of oxytocin to the decision for cesarean section is accounted for by variations in the value of the time from start of oxytocin to last dose change.

<4.0 cm dilated after the start of oxytocin because they are less dilated regardless of the route of delivery; dilatation of ≥ 4 cm is of no import. Segmenting the data by approximate "quartiles" of the maximum dose of oxytocin further diminishes the significance of the quantity of cervical dilatation (Table VIII). The size of the dose has a profound influence on the hours required to reach the final dose but no effect on the duration of the period that follows the last dose. Although the hours from the start of oxytocin to the final change of dose vary most significantly with the "quartiles" of the maximum dose, there is no difference in cervical dilatation for the cesarean section group. Dilatation was greater in the vaginal delivery patient group that received only 1 mU/min than in those patients who were treated with larger amounts of oxytocin.

Multiple regression analysis was used in a further attempt to determine what factors cause the duration of treatment and observation to vary in length. The variables used were: (1) elapsed time from the last examination before the start of oxytocin to complete dilatation or decision for cesarean section segmented into four groups (<mean - 1 SD, mean - 1 SD to mean, mean to mean + 1 SD, >mean + 1 SD); (2) the observation period, segmented similarly; (3) the time to select the maximum dose, as a continuous variable; (4) stopping or not stopping the oxytocin; (5) cervical di-

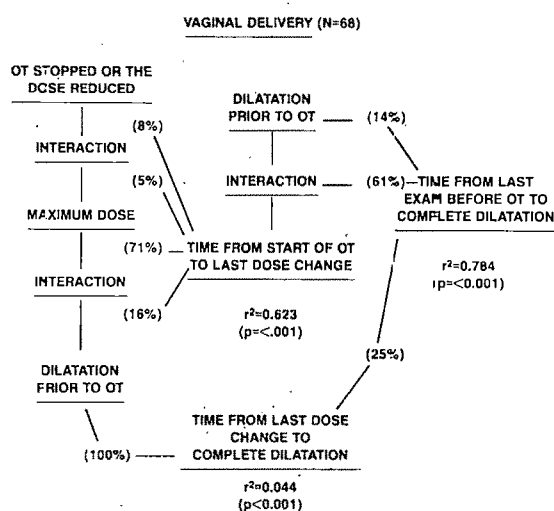


Fig. 3. Multiple regression analysis of the variance in: (1) elapsed time from last examination before oxytocin to complete dilatation, (2) elapsed time from initiation of oxytocin to last dose change in first stage, and (3) difference between them. r^2 is the proportion of the variance in the dependent variable that is explained by the independent variables. Values in parentheses are the percentage of the variation accounted for by that variable. For the explanation of *interaction*, see text.

latation before the start of oxytocin; (6) maximum dose of oxytocin; and (7-16) all pairwise combinations (interactions) of the independent variable. For the cesarean section group (Fig. 2), 82% (r^2) of the variance in the time from the last examination before oxytocin to the decision for cesarean section can be accounted for by fluctuations in the values for the durations of its component parts. The time to find the appropriate dose is the most important, accounting for 82% of the total time variance, whereas the observation period is responsible for only 18% of the explained variance. The proportion of differences in values of the maximum dose-finding period that can be explained is 79% (r^2), and two thirds is the result of differences in the maximum dose and only one third is accounted for by stopping the drug temporarily or reducing the dose. No factors could be identified to be responsible for differences in the "observation" period. Cervical dilatation before the start of oxytocin played no identifiable influential role in the duration or any of these time segments.

The results of the multiple regression study of the patients who were delivered vaginally present a contrast to those of the cesarean section group (Fig. 3). Seventy-eight percent of the variation in the total time after the start of oxytocin can be explained, and the degree of cervical dilatation before the start of oxytocin plays a significant part, responsible for 14% directly and 61% via its interaction with the maximum dose-selection period values. It is also the only identifiable factor ac-

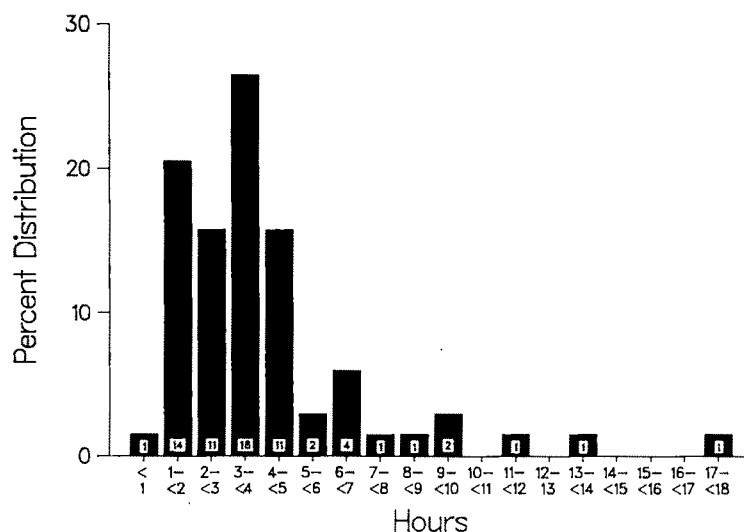


Fig. 4. Cervical dilatation time. The percentage distribution of duration of time from last notation of quantity of cervical dilatation before start of oxytocin or from time of start of oxytocin, whichever came later, to complete dilatation. The numbers of patients in each class interval are listed within the black bars.

counting for that small part of the variance in the observation period (100% of 4.5%) that can be explained. The maximum dose of oxytocin is important also, accounting for 71% of the explained variability (62%) in the time to select the maximum dose.

In a further search for evidences of amateurish management, the six patients whose elapsed time from the last examination before the start of oxytocin to the decision for cesarean section was more than 1 SD less than the mean, approximately <5 hours, are reported. A contracted pelvis was found in two, one being 54 inches tall. In another, low posterior implantation of the placenta was determined to be the cause of an inadequate response to oxytocin treatment. A fourth patient, 43 weeks pregnant, with preeclampsia and a small for gestational age fetus, was delivered abdominally when late decelerations were manifest 1.75 hours after the start of oxytocin. The remaining two patients had oxytocin therapy for <2 hours, after cessation of dilatation for many hours. The data suggest that only two of 33 cesarean sections (6%) might have been avoided.

We examined the overall rate at which the oxytocin was increased [(maximum dose minus the initial dose)/(time from the start of oxytocin to the last dose change)]. Although the patients delivered by cesarean section received the larger mean maximum dose, there was no significant difference in the overall mean rate of oxytocin incrementation in patients who had their initial dose increased: 1.2 ± 1.3 mU/min/hr in 29 of 33 patients in the cesarean section group and 1.5 ± 0.8 in 49 of 68 patients who were delivered vaginally. More than half of the patients in each group had oxytocin increased at an overall rate of 1 mU/min in more than

40 minutes. The size of the maximum dose of oxytocin had no effect on this measure of incrementation rate. The slow rate of increase in oxytocin dose may be a manifestation of the intermittent rather than the continuous presence of professional bedside attendants, but no differences in these respects are manifest between the cesarean and vaginal delivery groups.

In perusing the cervical dilatation rate data, we observed that many patients who were delivered vaginally seemed to have very rapid dilatation during fairly short periods of time. The frequency of such events is not germane to the issue of possible differences in the management of patients who are delivered vaginally or by cesarean section, but is relevant to our notions of the speed of dilatation characteristic of the successful response to oxytocin. To this end, we examined the time from the last notation of the quantity of cervical dilatation before the start of oxytocin or from the time of the start of oxytocin, whichever came later, to complete dilatation. This is the interval of time during which dilatation was identified after the start of oxytocin (Fig. 4). The average time required was 4.0 ± 2.9 hours; the shortest was 0.8 hour and the longest, 17 hours. The data are skewed by a few long values; 90% are less than 7 hours, and 75% <4.5 hours. Multiple regression analysis was used to identify those factors that might lengthen or shorten the dilatation periods for the 49 patients who received a maximum dose >1 mU/min and had the initial dose increased. The independent continuous variables tested were: cervical dilatation at the start of oxytocin, maximum dose of oxytocin, and overall mean rate of incrementation. The dichotomous ones were: the receipt or nonreceipt of magnesium

sulfate, and the stopping of oxytocin or its continuous administration. The proportion of the variance that can be explained is 43% (r^2), of which 32% is accounted for by magnesium sulfate use, 35% by the interaction of the maximum dose of oxytocin with the use of magnesium sulfate, and 33% by the interaction of the use of magnesium sulfate with the amount of cervical dilatation. The important influence of magnesium sulfate administration on the duration of the dilatation time in all 68 patients is reflected in the means: 3.2 ± 1.9 hours for the 46 patients who received no magnesium sulfate and 5.5 ± 4.0 hours for the 22 who did ($p = 0.002$). The cervical dilatation values before oxytocin were segmented by class intervals of <4 , 4 to 4.5, 5 to 5.5, 6 to 6.5, and 7 to 9.5 cm and the mean dilatation time was calculated for each bin. The group <4 cm dilated averaged 4.7 ± 1.8 hours and that of 7 to 9.5 cm, 2.2 ± 1.2 hours; each of these two class intervals was modestly but significantly different from the other four ($p = 0.037$ by one-way analysis of variance).

There were eight patients who received oxytocin in the first stage, had full dilatation, failed to achieve a station suitable for vaginal birth, and were delivered by cesarean section. This group is too small for serious analysis. However, there was no significant difference between the cesarean section patients who had complete dilatation and those who did not in the following variables: mean cervical dilatation before oxytocin, maximum dose of oxytocin, elapsed time from the last examination to the decision for cesarean section, time from the start of oxytocin to the last dose change, or period of observation after the last dose change.

Comment

The patients who were delivered by cesarean section were disadvantaged at admission by lesser cervical dilatation than that in those delivered vaginally. The higher frequency in the cesarean section group of pre-eclamptic patients admitted at their first visit to the labor-delivery suite probably accounts for the difference in dilatation. This inequality was dissipated by the time of amniotomy. The cesarean section patients received every opportunity to be delivered vaginally. They were given larger doses of oxytocin for longer periods of time. After the last dosage change they were observed for a period of time, equivalent to that required by the vaginal delivery patients to develop full dilatation, before the decision for cesarean section was made. Management phenomena such as the frequency of examination and the rate of increase of the oxytocin dose were the same in both groups.

The data were examined for evidences of amateurish care because the details of management after the decision to use oxytocin and before the decision to perform a cesarean section are the responsibility of junior residents. Only one of 33 patients had a cesarean section because of an abnormal fetal heart rate in addition to failure of the cervix to dilate in response to oxytocin therapy. Only one of 33 cesarean section patients developed intra-amniotic infection. The slow rate of increase in oxytocin dose in those patients who received more than the initial dose is inefficient care and probably prolongs the patients' labor room experience unnecessarily, but both the patients who were delivered vaginally and those delivered by cesarean section were treated similarly in this respect. This "dawdling" dose-incrementation regimen probably results from our system of care characterized by intermittent visits for the purpose of assessment of the oscillographic record of the patient's progress by residents who have additional labor-delivery suite duties. This belief is supported by the fact that 23% of our patients who were delivered vaginally required more than 12 hours from admission to delivery; this can be compared with the low incidence observed when continuous bedside nursing is provided for the evaluation of the quality of labor.⁴

We conclude that the decision to perform cesarean section in nulliparous patients with functional dystocia in the first stage of labor treated by amniotomy and oxytocin arises from disabilities of the patient and is not the result of differences in the application of our patient management principles, services, or treatments. Rather we believe the result obtains from the interaction of our patients, who have little education and a high incidence of obstetric abnormalities such as pre-eclampsia, with a professional care cadre of nurses, pediatricians, and obstetricians who expect to effect progressively decreasing perinatal mortality and morbidity rates.

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Maternal serum α -fetoprotein screening: Low and high values for detection of genetic abnormalities

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In initiating a maternal serum α -fetoprotein screening program, we pursued not only elevated values for the detection of neural tube defects but also low values to detect trisomic fetuses. We detected neural tube defects (0.2%) as expected but were surprised by the efficacy with which low serum α -fetoprotein values identified aneuploid fetuses. In 1421 pregnant women, 132 (9.3%) showed maternal serum α -fetoprotein values <0.4 multiple of the median. After repeat sampling, 57 women still had low values. These 57 women and six others who were too anxious for repeat sampling underwent level I ultrasound examination with the following results: gestational age overestimated by 2 weeks ($n = 8$), fetal death ($n = 1$), and no explanation ($n = 54$). Of the 54, 49 underwent amniocentesis with detection of three aneuploid fetuses: trisomy 18 ($n = 2$) and trisomy 21 ($n = 1$); maternal ages were 27, 29, and 31 years, respectively. Autosomal aneuploidy did not occur in other women screened. We conclude that low maternal serum α -fetoprotein values could efficiently detect aneuploid fetuses, perhaps with greater sensitivity than previously predicted. (AM J OBSTET GYNECOL 1986;155:593-7.)

Key words: Maternal serum α -fetoprotein, neural tube defects, trisomic fetuses

Maternal serum α -fetoprotein is accepted as an efficient way of detecting neural tube defects. In the United States, however, the propriety of introducing maternal serum α -fetoprotein screening programs depends on the ability of the public and medical community to handle problems that may arise. Approximately 3% to 7% of women will show "elevated" values, the precise figure depending on the specific population and on the arbitrarily chosen values above which an elevation is said to exist. However, most women with elevated maternal serum α -fetoprotein values do not have a fetus with a neural tube defect. If 5% of pregnant women show elevated maternal serum α -fetoprotein values, only one of 25 (0.2% of original sample) such women will actually have a fetus with a neural tube defect.¹

Despite some earlier reservations,² it is increasingly evident that potential problems can be managed. With the availability of commercial reagents, one potential stumbling block was removed. A further advance in-

creasing the desirability of maternal serum α -fetoprotein screening was the observation that *low* maternal serum α -fetoprotein values are correlated with aneuploidy.^{3,4} For all the above reasons, maternal serum α -fetoprotein now seems reasonable. Of course, screening should remain voluntary, at the discretion of the patient. In this communication we shall describe our initial experience with a maternal serum α -fetoprotein screening program in which we acted on both low and high values.

Methods and material

In 1984, the Section of Human Genetics, Department of Obstetrics and Gynecology, and the Clinical Immunology laboratory, Department of Pathology, jointly initiated a maternal serum α -fetoprotein screening program. In order to minimize problems it was decided that all specimens would be coordinated through a genetic counselor located in the Section of Human Genetics. This person was responsible for logging specimens, relaying results to referring physicians, coordinating repeated blood samples, arranging ultrasonography or amniocentesis appointments, and offering genetic counseling.

Assay verification. Samples are either delivered personally or mailed. We recommend separating serum if samples are mailed or stored overnight. However, we have observed no alterations in maternal serum α -fetoprotein values if serum is stored overnight or even frozen.

The three approved commercial reagents were com-

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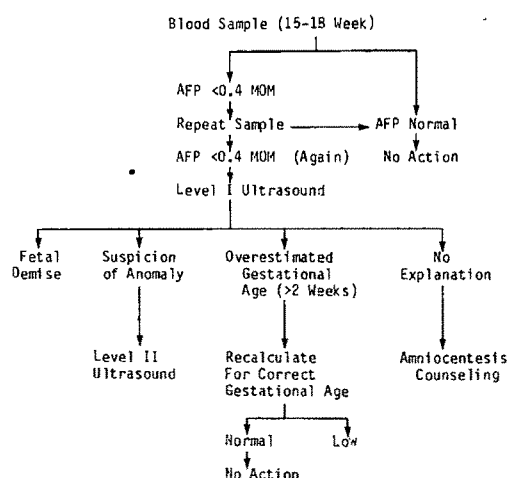


Fig. 1. Protocol used at Northwestern University for maternal serum α -fetoprotein protein (AFP) screening. MOM = Multiple of the median.

pared. Specifically, we tested sera of at least 25 women per week, at gestational weeks 15 to 20. The test kit showing the lowest coefficient of variability proved to be the Kallestad AFP/OB radioimmunoassay kit (Kallestad Laboratories, Inc., Austin, Texas). Our values proved quite similar to those of the manufacturer. In our hands, the assay gave interrater coefficients of variation of 7.2%. Thereafter, a given sample was considered elevated if it was above 2.5 multiples of the median as defined by either our (Northwestern) laboratory or by the manufacturer. In some cases this may have led to a lower multiple of the median than was actually the case. Our normal values are shown in Table I. We chose not to correct for maternal weight, considering data verifying its necessity to be unavailable.

Nonetheless, maternal serum α -fetoprotein assays display a moderate degree of variation. The precision of the cutoff values at the upper as well as lower limits of normal must be viewed with some caution.

Maternal serum α -fetoprotein was considered elevated if the value was >2.5 multiples of the median for the appropriate gestational age (Table I). Detection of an elevated value necessitated repetition, although occasionally this step was bypassed by a referring obstetrician if (1) a value was very elevated or (2) the pregnancy was quite advanced. If a second sample similarly proved elevated, level I ultrasonography was recommended. Should ultrasonographic assessment (biparietal diameter, femur length) reveal the gestational age to be greater than 2 weeks over that expected, a valid explanation for the elevated maternal serum α -fetoprotein value was considered to exist (provided, of course, that the value was normal for the newly determined stage of gestation). (A 2-week discrepancy was

Table I. Normal laboratory values at Northwestern

Gestational age (wk)	Maternal serum α -fetoprotein (IU/ml)		
	Median*	2.5 MOM	0.4 MOM
15	16.4	41.0	6.6
16	26.3	65.8	10.5
17	28.6	71.5	11.4
18	29.5	73.8	11.8
19	28.5	71.3	11.4
20	41.0	102.5	16.4

MOM = Multiple(s) of the median.

*Kallestad radioimmunoassay.

chosen because that interval is beyond the standard error of ultrasonic measurements.) Twins were also considered a valid explanation, provided maternal serum α -fetoprotein values did not exceed five times the median for a single pregnancy.

If two elevated maternal serum α -fetoprotein values were observed without explanation, then women were counseled that neural tube defects or other anomalies could exist. The likelihood of neural tube defect was stated to be 1 in 10. Although level II ultrasonography alone was offered as one option, patients were informed that amniocentesis was the more definitive procedure to exclude neural tube defects.

Follow-up of low maternal serum α -fetoprotein. A maternal serum α -fetoprotein value <0.4 multiple of the median was arbitrarily chosen as a value below which follow-up was necessary. Since low maternal serum α -fetoprotein screening programs had not actually been implemented, the propriety of obtaining a second sample (as for elevated values) was uncertain. However, we considered it logistically preferable for low and high maternal serum α -fetoprotein values to be followed up in comparable fashion. Thus a second sample was requested if a value <0.4 multiple of the median was obtained. If the second serum value also proved decreased, level I ultrasound to exclude underestimated gestational age was recommended (Fig. 1). (Again, a 2-week interval was accepted as a valid explanation for an ostensibly low maternal serum α -fetoprotein.) If gestational age was correct and if fetal viability was confirmed, the likelihood of aneuploidy was stated to be increased over that expected for maternal age. The magnitude of the increase was said to be "uncertain" but estimated at threefold to sevenfold. Benefits and risks of amniocentesis were discussed.

Results

Elevated maternal serum α -fetoprotein values. As of September 1985, 1688 women had been screened. Of these, 55 (3.3%) showed a maternal serum α -fetoprotein elevation >2.5 multiples of the median for a

given gestational interval (Fig. 2). Nine went directly to Level I ultrasound. Of the 45 in which a second sample was submitted, 29 (65%) again showed an elevated value. Overall, 39 women with one or more elevated maternal serum α -fetoprotein values were offered level I ultrasound with the outcomes listed in Fig. 2: four multiple gestations, one fetal demise, two anomalies evident on level I ultrasound (one cystic hygroma and one anencephaly), and 29 unexplained. In the 29 unexplained cases, 24 underwent amniocentesis. One meningomyelocele was detected and confirmed by level II examination; elective terminations were chosen.

We are not aware of neural tube defects occurring among those women having only normal maternal serum α -fetoprotein values. Of interest is that no neural tube defects occurred among women whose initial maternal serum α -fetoprotein was elevated but whose second value was normal. In all these cases, the initial values had been only slightly greater than 2.5 multiples of the median.

Low maternal serum α -fetoprotein values. A total of 1421 women were screened because of low maternal serum α -fetoprotein values. (This part of our program began several months after that for elevated maternal serum α -fetoprotein values; thus the sample was slightly smaller than that screened for elevated values.) Of 1421 samples, 132 (9.3%) showed values <0.4 multiple of the median (Fig. 3). Of these, 116 repeat samples were submitted, with 57 (49%) still <0.4 multiple of the median. In 10 of the 132, women elected neither repeat maternal serum α -fetoprotein determination, ultrasound, nor amniocentesis; six of these 10 had previously undergone chorionic villi sampling with a normal fetal karyotype. The 59 women showing only a single low value manifested no apparent increase in fetal deaths. However, detailed comparisons have not yet been made. The group contained no autosomal trisomies.

Fifty-seven women having two consecutive low values and six women having only one low value underwent level I ultrasonography. One of the 63 had a fetal death. Nine of these 63 had erroneous dates (overestimated by ≥ 2 weeks); all but one had normal maternal serum α -fetoprotein for the true corrected gestational age. In 53 other women there was no explanation for the low values. When the 53 women with unexplained values were counseled as described, 48 chose amniocentesis. Three trisomic fetuses were detected (two trisomy 18; one trisomy 21). Their maternal ages were 27, 29, and 31 years, respectively.

We are aware of no autosomal trisomies in those 1234 women having only normal maternal serum α -fetoprotein values or in the nine who declined amniocentesis after showing one or more low values. However, not all have been delivered, and it is impractical to perform

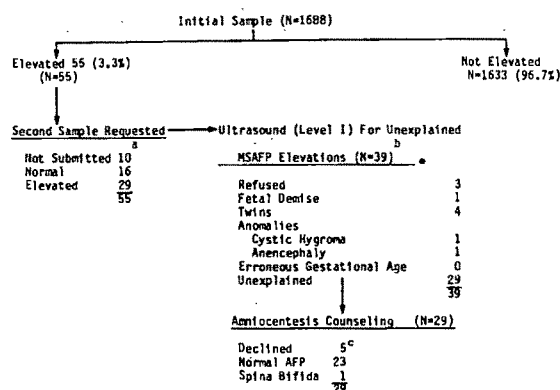


Fig. 2. Outcome of elevated (>2.5 multiples of the median) maternal serum α -fetoprotein (MSAFP) values. *a* = Ultrasound directly ($n = 9$); follow-up refused ($n = 1$). *b* = After two elevations ($n = 29$); after one elevation ($n = 10$). *c* = Level II ultrasound normal ($n = 5$).

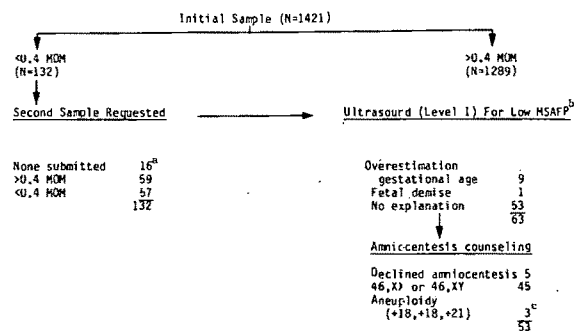


Fig. 3. Outcome of low (<0.4 multiple of the median, MOM) maternal serum α -fetoprotein (MSAFP) values. *a* = Cases not resubmitted because chorionic villus sampling was previously performed ($n = 6$); cases went directly to Level I ultrasound ($n = 6$); cases not desiring follow-up ($n = 4$). *b* = Cases after two low values ($n = 57$); cases after only one low value ($n = 6$). *c* = Maternal ages 27, 29, and 31 years.

chromosomal studies of all newborn infants (the ideal experimental design).

Comment

Initiation of maternal serum α -fetoprotein screening at Northwestern University appeared to be accomplished with minimal confusion on behalf of the primary provider, coordinating genetics unit, and laboratory. Moreover, findings with respect to serum maternal serum α -fetoprotein values were not unexpected. One surprise in screening elevated maternal serum α -fetoprotein values was the rarity with which fetal death or erroneous dates explained elevated values. Among 29 elevated values, we observed no erroneous dates and only one fetal death. These findings presumably reflect frequent use of ultrasound in our referral population. Overall, we detected two neural tube defects (0.2% initial sample), about the expected

population incidence. One cystic hygroma and four sets of twins were also detected.

Our findings are similar to those of larger series of other investigators. Using 2.0 multiples of the median as an upper limit of normal, Macri and Weiss⁵ detected 20 open neural tube defects among 17,703 pregnancies (0.12%); two cases were missed, for a sensitivity of 91%. Also using 2.0 multiple of the median as the upper limit, Haddow et al.⁶ detected six of nine cases of open neural tube defects among 3636 pregnancies; two of their three missed cases were in discordantly affected twin pregnancies. Although a 1977 United Kingdom maternal serum α -fetoprotein collaborative study predicted that 88% of open neural tube defects could be detected with the ninetieth percentile used as the upper cutoff, only 77.8% of open neural tube defects were actually detected in more than 15,000 pregnancies screened.⁸ In North Carolina, Burton et al.⁹ used a higher cutoff of 2.5 multiples of the median to detect 15 of 18 open neural tube defects, a detection rate of 83% in 12,084 patients. (The advantage of using a higher cutoff is that fewer false positive values exist; therefore, fewer patients undergo needless genetic amniocentesis. The theoretical cost is lower sensitivity.)

Not anticipated when our program was originally planned was the usefulness of low maternal serum α -fetoprotein values. Originally reported by Merkatz et al.,³ the association of low maternal serum α -fetoprotein values with aneuploidy has received increasing attention. Retrospectively analyzing the United Kingdom maternal serum α -fetoprotein data, Cuckle et al.⁴ calculated that maternal serum α -fetoprotein values <0.4 multiple of the median increased the likelihood of Down syndrome threefold over that of a given maternal age. Fuhrmann et al.¹⁰ estimated the increase to be sevenfold. Martin and Liu¹¹ provide tables useful for counseling.

Among 48 amniocenteses performed for low (<0.4 multiple of the median) maternal serum α -fetoprotein, three trisomic fetuses were detected. The yield (6.3% amniocenteses) is greater than that of amniocentesis in women 35 or 36 years of age. Still, many issues remain concerning the proper way to assess and pursue low values. In this respect a few comments are in order concerning our own program. First, we chose 0.4 multiple of the median as the value below which the maternal serum α -fetoprotein value was said to be low. It is actually not known, however, whether the risk of aneuploidy continues to increase as the maternal serum α -fetoprotein value approaches zero. Does 0.2 multiple of the median carry a greater risk than 0.4 multiple of the median? Second, we chose to obtain a second sample when maternal serum α -fetoprotein was <0.4 . Follow-up for a low maternal serum α -fetoprotein value was thus analogous to that for an elevated value.

Among the 57 women having a value that was initially low but later >0.4 multiple of the median we detected no autosomal trisomies. However, the propriety of repeating samples is still unproved for low maternal serum α -fetoprotein. Third, we attributed low values to erroneous gestational age only if the discrepancy between assigned gestational age and ultrasound was 2 weeks. This is the same interval required to correct an elevated value. Fourth, we did not correct for fetal weight or maternal weight yet still detected aneuploidy. Fifth, we avoided precise cutoffs, with respect to offering amniocentesis. Instead, we provided risks and performed amniocentesis if couples desired. This contrasts with proposals by others to use a combination of maternal serum α -fetoprotein values and maternal age to define precisely those circumstances under which amniocentesis is appropriate.^{12,13} Finally, we believed it premature to consider elevated serum α -fetoprotein values as protective against aneuploidy.

The mechanism responsible for the relationship between maternal serum α -fetoprotein values and autosomal trisomy is unknown. One explanation could be decreased production of α -fetoprotein by trisomic fetuses. Indeed, some investigators^{14,15} have shown decreased amniotic fluid α -fetoprotein values in trisomy 21 pregnancies. The three trisomic pregnancies detected by our maternal serum α -fetoprotein screening program all showed amniotic fluid α -fetoprotein values below the mean for their gestational age (trisomy 18 fetuses, 0.2 and 1.3 SD below the mean; trisomy 21 fetus, 1.4 SD below the mean). If trisomic fetuses indeed produce less α -fetoprotein than normal fetuses, what is the pathogenesis? Decreased fetal weight is one possibility, in view of the well-known association between intrauterine growth retardation and autosomal trisomy. However, the fetus weighs only 150 to 200 gm at 16 weeks' gestation, and differences in weights between aneuploid and euploid fetuses might not be large enough to explain differences in maternal serum α -fetoprotein values. Comparing birth weights of euploid infants whose mothers do or do not show decreased maternal serum α -fetoprotein values would help resolve this issue. Another possibility is that trisomic fetuses are immature compared with euploid fetuses, manifesting a maturational delay in liver development. Low serum α -fetoprotein values could also reflect a specific cellular abnormality inherent in trisomic cells.

In this communication we have considered only the issue of anomaly detection through maternal serum α -fetoprotein screening. Detection of other obstetric complications is possible, as emphasized by our not infrequent detection of twins. In particular, intrauterine growth retardation, abruptio placentae, and other abnormalities are associated with persistently elevated maternal serum α -fetoprotein values in pregnancies

without neural tube defects. These and other correlations with low and high maternal serum α -fetoprotein values may prove clinically useful.

Addendum

Knight et al. (N Engl J Med 1986;314:516) recently noted a tendency for the Kallestad radioimmunoassay to provide a high proportion of low maternal serum α -fetoprotein values. The 9.3% low values we observe are consistent with comparisons of Knight and Haddow. However, our detection of three trisomies makes their suggestion of avoiding the Kallestad assay for low maternal serum α -fetoprotein testing at least arguable.

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α -Fetoprotein, human placental lactogen, and pregnancy-specific β_1 -glycoprotein in pregnant women who drink: Relation to fetal alcohol syndrome

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To determine the usefulness of the assays of maternal serum α -fetoprotein, human placental lactogen, and pregnancy-specific β_1 -glycoprotein in prenatal diagnosis of fetal alcohol syndrome, these proteins were followed in 35 problem drinkers (>100 gm of ethanol weekly) and in 14 abstinent women throughout gestation. Thirteen women gave birth to infants with fetal alcohol syndrome, and these women had low levels of α -fetoprotein and pregnancy-specific β_1 -glycoprotein but levels of human placental lactogen were normal. Low α -fetoprotein correctly predicted fetal alcohol syndrome in 59% with a relative risk of 2.46. Low pregnancy-specific β_1 -glycoprotein predicted fetal alcohol syndrome in 56%, and a relative risk was 3.29. Low α -fetoprotein and pregnancy-specific β_1 -glycoprotein may reflect primary or secondary effects of ethanol abuse in pregnancy and appear to be useful in predicting fetal alcohol syndrome. (AM J OBSTET GYNECOL 1986;155:598-601.)

Key words: α -Fetoprotein, human placental lactogen, pregnancy-specific β_1 -glycoprotein, maternal drinking, fetal alcohol syndrome

No prenatal diagnostic measures exist for fetal alcohol syndrome (prenatal and/or postnatal growth retardation, facial characteristics, neurological aberrations), one of the most common fetal anomalies encountered today.¹ In this regard, α -fetoprotein may be of interest, because it is synthesized in fetal liver, which becomes exposed to ethanol in drinking women.² Furthermore, it is well established that serum α -fetoprotein is often elevated in pregnant women with fetal death or anomalies (for example, intrauterine deaths, neural tube defects, intestinal atresias, renal malformations).³ Moreover, placental proteins, such as human placental lactogen and pregnancy-specific β_1 -glycoprotein, which are commonly employed for the biochemical assessment of placental function,⁴ might show abnormalities, particularly because ethanol may directly damage the placenta of drinking women.⁵ Because no information is available about the concentrations of α -fetoprotein, human placental lactogen, and pregnancy-specific β_1 -glycoprotein in pregnant problem drinkers, we followed their concentrations throughout the pregnancy and related them to fetal outcome.

Material and methods

Forty-two patients were referred by local midwives to a special outpatient department for drinking pregnant women between the ninth and twenty-fourth weeks of gestation. After the first alcohol counseling at registration and plea for abstinence during pregnancy, the drinkers were encouraged to visit the department at 2 to 4 weeks' intervals for additional counseling and control. However, 35 women continued to drink alcohol, as evident from their own reports (weekly consumption at least 100 gm of pure ethanol), from reports of nurses at the local antenatal care centers, and/or from determinations of ethanol in blood and urine at each visit, and they were included in the present work. We also recruited 14 abstinent women from the same region who were similarly followed at the same research center (Table I). The drinking and nondrinking groups were comparable in age, parity, and weight, but the drinkers smoked somewhat more commonly (77%) than controls (30%) (Table I). One of the drinkers was epileptic and used 300 mg of carbamazepine daily. In another one the right ovary had been removed because of a semimalignant ovarian tumor 1 year before pregnancy, but no recurrence or metastasis had occurred. No drinker or control developed hypertensive or cholestatic pregnancy complications.

Thirteen drinkers (37%) gave birth to infants with fetal alcohol syndrome, one of whom was stillborn (Table I). The diagnosis was based on the following criteria: intrauterine growth retardation (birth weight

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Table I. Clinical characteristics of the study populations (mean $\pm$ SD)

	Women who drank		Control subjects (n = 14)
	Infant with fetal alcohol syndrome (n = 13)	Healthy infant (n = 22)	
Maternal age (yr)	31.0 $\pm$ 6.3	29.4 $\pm$ 5.3	28.1 $\pm$ 5.4
Parity	1.8 $\pm$ 1.0	1.8 $\pm$ 1.1	1.9 $\pm$ 0.8
Weight before pregnancy (kg)	63.1 $\pm$ 14.1	60.5 $\pm$ 9.0	56.7 $\pm$ 7.2
Smoking (%)	70	80	30
Gestational age at delivery (wk)	38.7 $\pm$ 2.0	39.8 $\pm$ 1.7	39.3 $\pm$ 2.0
Birth weight (gm)	2702 $\pm$ 471*†	3203 $\pm$ 352*	3420 $\pm$ 390†
Placental weight (gm)	529 $\pm$ 94*	572 $\pm$ 89	643 $\pm$ 95*

*p < 0.05.

†p < 0.005.

Table II. The sensitivity, specificity, predictive value, and relative risk of low α -fetoprotein, human placental lactogen, and pregnancy-specific β_1 -glycoprotein in predicting fetal alcohol syndrome

	α -Fetoprotein	Human placental lactogen	Pregnancy-specific β_1 -glycoprotein
Sensitivity (%)	43	7	60
Specificity (%)	85	93	77
Predictive value (%)	59	33	56
Relative risk	2.46	1.06	3.29

below the tenth percentile; nine of 13 subjects), post-natal growth retardation (weight and/or height and/or head circumference <2 SD for age; eight of 12), neurological aberrations (10 of 12, with two having only hypotonia), and facial characteristics (12 of 12). In addition, two infants had bilateral clubfoot and one had an extra thumb. One of the infants of drinkers had esophageal atresia and one had unilateral clubfoot but no typical fetal alcohol syndrome; they were not included in the fetal alcohol syndrome group. All the infants born to the control women were healthy. The follow-up extended to at least 6 months for each infant.

Venous blood samples were drawn at each follow-up visit. Serum was separated with centrifugation and stored frozen (-20°C) until assayed for α -fetoprotein and pregnancy-specific β_1 -glycoprotein as described before,^{6,7} or for human placental lactogen, with use of a commercial radioimmunoassay kit (Nordiclab, Oulu, Finland). All the samples were assayed in the same batch. The interassay variation was between 13% to 15% and the intra-assay variation between 11% to 14%.

The normal ranges (between the tenth and ninetieth percentiles) for α -fetoprotein, human placental lactogen, and pregnancy-specific β_1 -glycoprotein were calculated from the data of the abstinent women. Because a comparison with the controls (Mann-Whitney test) revealed that α -fetoprotein and pregnancy-specific β_1 -glycoprotein were reduced in drinkers, the low levels of these markers (individual measures below the tenth percentile) were considered in relation to fetal alcohol syndrome as follows: true positive (TP) (test low, fetal

alcohol syndrome positive), false positive (FP) (test low, fetal alcohol syndrome negative), true negative (TN) (test normal, fetal alcohol syndrome negative), and false negative (FN) (test normal, fetal alcohol syndrome positive). Sensitivity of the indices was $TP/(TP + FN)$, specificity was $TN/(TN + FP)$, predictive value was $TP/(TP + FP)$, and the relative risk factor was the ratio of $TP/(TP + FP)$ to $FN/(FN + TN)$. The significance of the differences between the percentages was studied by binomial *t* test.

Results

Seventy-four percent of the α -fetoprotein concentrations in drinkers with healthy infants fell within the normal range (Fig. 1). The variation was larger if the infant had fetal alcohol syndrome, and the highest values were encountered in the woman with previous semimalignant ovarian tumor (Fig. 1). However, nine of the 13 subjects with fetal alcohol syndrome and nine of the 22 drinkers with healthy infants (69% and 41%, respectively; $p < 0.05$) had one or fewer measures below the tenth percentile. The majority of the measures (43%) of the subjects with fetal alcohol syndrome were low (Table II). Low α -fetoprotein predicted fetal alcohol syndrome correctly in 59% with a relative risk of 2.46 (Table II).

The levels of human placental lactogen were not affected by maternal drinking (Fig. 2), and occurrence of fetal alcohol syndrome could not be predicted by human placental lactogen assay (Table II).

Maternal drinking tended to be accompanied by re-

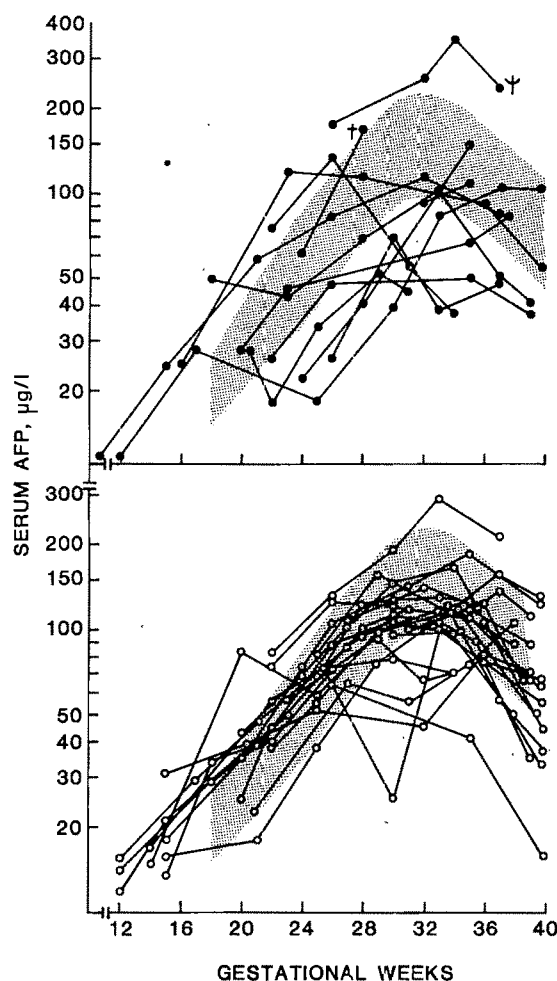


Fig. 1. Concentrations of α -fetoprotein in drinking mothers. Shaded area indicates the normal tenth and ninetyth percentiles. ● = Mothers of infants with fetal alcohol syndrome. ○ = Mothers who drink and have healthy infants. † = Fetal death. ψ = Previous semimalignant ovarian tumor.

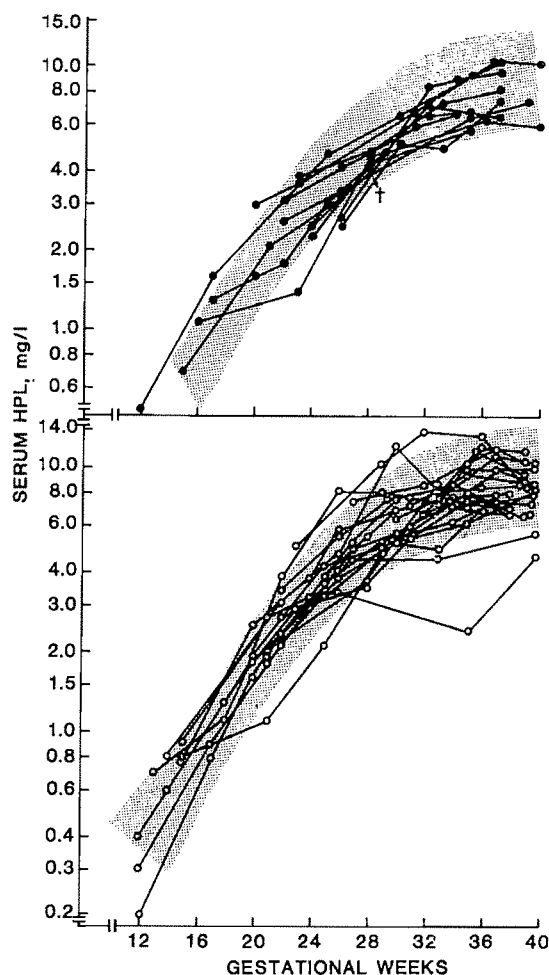


Fig. 2. Concentrations of human placental lactogen in drinking mothers. Shaded area indicates the normal tenth and ninetyth percentiles. ● = Mothers of infants with fetal alcohol syndrome. ○ = Mothers who drink and have healthy infants. † = Fetal death.

duced pregnancy-specific β_1 -glycoprotein levels from the twentieth gestational week onward (Fig. 3). This reduction was most marked if the infant had fetal alcohol syndrome (Table II), and in fact, 12 of these 13 infants had at least one measure below the tenth percentile. As only nine of the 22 drinkers with healthy infants had one or fewer pregnancy-specific β_1 -glycoprotein measures below the tenth percentile, the difference between the groups was significant ($p < 0.0005$). The relative risk of fetal alcohol syndrome was 3.29 in drinking women with low pregnancy-specific β_1 -glycoprotein, which had a predictive value of 56% (Table II).

Three of four women with fetal alcohol syndrome, who had normal α -fetoprotein levels, had at least one subnormal level of pregnancy-specific β_1 -glycoprotein. Likewise, the woman with normal pregnancy-specific β_1 -glycoprotein levels through pregnancy also had nor-

mal α -fetoprotein levels. Thus the combined use of α -fetoprotein and pregnancy-specific β_1 -glycoprotein revealed fetal alcohol syndrome in all cases except in one with previous semimalignant ovarian tumor.

Comment

Although fetal alcohol syndrome and/or "fetal alcohol effects" are a common problem,¹ no previous efforts to predict them prenatally have been made. This may be partly due to difficulties in performing a prospective study on drinking women, who often totally neglect prenatal care. We have run a special outpatient department for pregnant problem drinkers since 1983, and this gave us an opportunity to evaluate the usefulness of α -fetoprotein, human placental lactogen, and pregnancy-specific β_1 -glycoprotein in the prenatal diagnosis of fetal alcohol syndrome.

All of our patients drank at least 100 gm of ethanol

weekly, and some of them consumed much more alcohol (up to the two bottles of vodka per day during the first half of pregnancy). Thirty-seven percent of these women delivered an infant with fetal alcohol syndrome, and they were characterized by low α -fetoprotein and pregnancy-specific β_1 -glycoprotein but normal human placental lactogen.

α -Fetoprotein, which is synthesized in the fetal liver, is present in high concentrations in the fetal circulation and amniotic fluid, wherefrom it is transferred through the placenta and/or membranes to the maternal blood.³ Basically, reduced α -fetoprotein in the drinkers with fetal alcohol syndrome may result either from decreased synthesis and/or transport to the maternal side or from increased elimination of α -fetoprotein from maternal blood. No data to support the latter possibility are available. Therefore we feel that ethanol and/or its metabolites may have decreased the synthesis and/or transport of α -fetoprotein and thus led to reduced levels in maternal serum. The low α -fetoprotein in women with fetal alcohol syndrome is not accounted for by the small size of the fetus or maternal smoking, as these factors are associated with elevated maternal α -fetoprotein.⁸⁻¹⁰ Curiously, the woman with a history of semimalignant ovarian tumor had the highest α -fetoprotein levels, although the infant had typical fetal alcohol syndrome. The high α -fetoprotein in this woman is probably not connected with the previous tumor because no recurrence was seen during cesarean section, which was performed because of breech presentation.

The placental syncytiotrophoblasts produce both human placental lactogen and pregnancy-specific β_1 -glycoprotein.⁴ Therefore it was of interest that these proteins behaved differently in the drinking women with fetal alcohol syndrome, since only pregnancy-specific β_1 -glycoprotein levels were reduced. No data suggest an increased metabolism and/or excretion of pregnancy-specific β_1 -glycoprotein in drinking women, and therefore it is conceivable that pregnancy-specific β_1 -glycoprotein synthesis was specifically reduced by ethanol and/or its metabolites in the heavy drinkers. It was also noteworthy that pregnancy-specific β_1 -glycoprotein levels in drinkers without fetal alcohol syndrome tended to be slightly reduced.

Low maternal α -fetoprotein and low pregnancy-specific β_1 -glycoprotein were about equally effective in the prediction of fetal alcohol syndrome. In view of the predictive values (59% to 56%) and relative risks (2.46 to 3.29), these tests can be recommended for routine use in the antenatal care of drinking women. If they are low and/or decreasing in serial determinations and strict abstinence cannot be achieved, the delivery of the infant should be considered as soon as fetal lung maturity can be confirmed. When, and if, the mechanisms

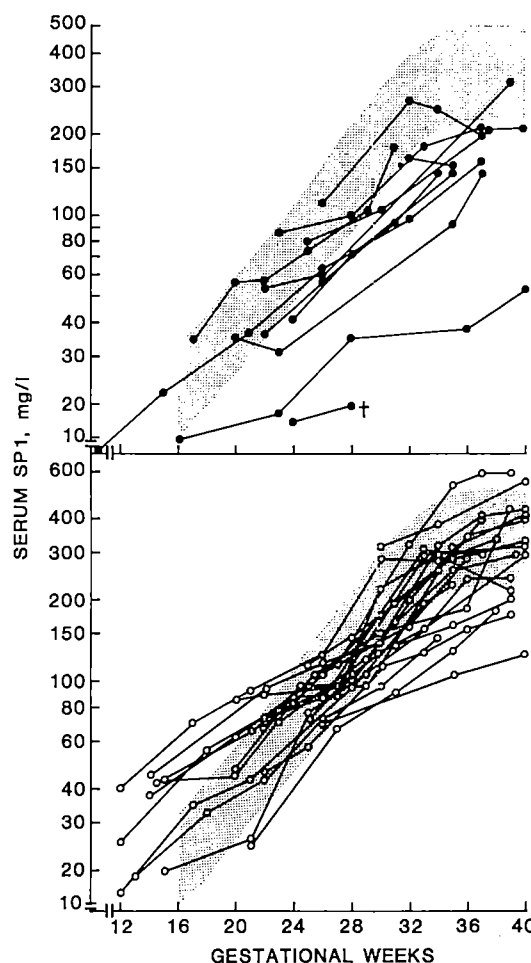


Fig. 3. Concentrations of pregnancy-specific β_1 -glycoprotein in drinking mothers. Shaded area indicates the normal tenth and ninetieth percentiles. ● = Mothers of infants with fetal alcohol syndrome. ○ = Mothers who drink and have healthy infants. † = Fetal death.

by which ethanol damages the fetus are understood in the future, low α -fetoprotein and/or pregnancy-specific β_1 -glycoprotein in drinking women may lead to additional measures to prevent or ameliorate the development of fetal alcohol syndrome.

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Lower genital tract infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Icelandic women with salpingitis

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In a study of 225 women with acute salpingitis verified by laparoscopy or laparotomy we investigated the prevalence of gonococcal and chlamydial infection in the lower genital tract. *Neisseria gonorrhoeae* was isolated from 18.9% of the women and *Chlamydia trachomatis* from 38.5%. Women with positive cultures were significantly younger ($p < 0.01$) than those with negative cultures. A trend toward more severe inflammatory changes of the tubes was found in women with positive cultures compared with those with negative cultures. The majority of women with positive cultures stated they had only one sexual partner during the preceding 6 months. Single women had more partners (mean 1.9) than those cohabiting (mean 1.2). The ratio of single/multiple partners for women with *Chlamydia* was 2.5:1, and for those with gonorrhea 1:1 ($p < 0.05$). Of the men, 60% could be examined and about 50% had positive cultures. Microbiologic investigation of both partners is necessary in order to prevent reinfection of the woman. (*AM J OBSTET GYNECOL* 1986;155:602-7.)

Key words: Salpingitis, gonorrhea, chlamydia infections, venereal diseases

Acute salpingitis is a serious condition that often leads to permanent tubal damage and infertility. In a majority of cases salpingitis is associated with sexually transmitted organisms, primarily *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.¹⁻³ The relative ease with which most sexually transmitted diseases are now cured contrasts with the serious problems resulting from salpingitis such as infertility, ectopic pregnancy, chronic abdominal pain, dysmenorrhea, dyspareunia, and psychological morbidity.^{4,6} In the past it has been difficult to evaluate the role of sexually transmitted infections in the etiology of salpingitis, because the clinical diagnosis of salpingitis was frequently inaccurate⁷⁻⁹ and suit-

able methods for the culture of sexually transmitted organisms have been lacking in many clinical laboratories. At the Department of Obstetrics and Gynecology, National Hospital, Reykjavik, Iceland, it has been policy for a number of years to verify all clinical diagnoses of salpingitis by laparoscopy.⁸ Improved culture techniques have also been instituted in an effort to obtain more accurate microbiologic identification. We report the findings from a study of the association between salpingitis and sexually transmitted microorganisms at our hospital.

Material and methods

Patients. The study population consisted of 225 consecutive cases of acute salpingitis verified by laparoscopy or laparotomy and treated in the Department of Obstetrics and Gynecology of the National Hospital, Reykjavik, Iceland, between January 1, 1982, and May 31, 1984. The criteria used for the diagnosis of salpingitis were those described by Weström⁶ and graded as follows: mild—tubes reddened, swollen, covered by pu-

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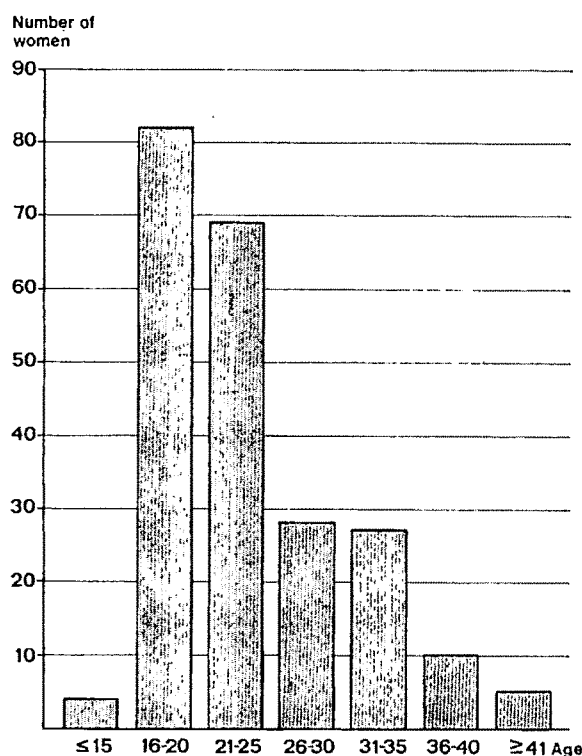


Fig. 1. Age distribution of women with acute salpingitis (n = 225).

ruent discharge, "violin-string" adhesions, or fibrin deposits but freely movable and with open abdominal ostia; moderate—same as for mild, but more marked changes, with tubes not freely movable and patency of abdominal ostia uncertain; severe—pelvic peritonitis and/or abscess formation, ostia closed. Clinical details concerning age, marital status, and severity of disease as well as microbiologic results were extracted from the hospital case records.

Sexual partners. In Iceland, gonorrheal and chlamydial infections are both notifiable as sexually transmitted diseases. When these are diagnosed at the Department of Obstetrics and Gynecology, our social workers interview the patient and obtain information on the patient's sexual partner(s) during the preceding 6 months. The particulars concerning the partner(s) are then relayed to the Venereal Diseases Clinic at the Reykjavik Health Centre, from which they are contacted and treated if necessary.

Data on the partners of women with positive cultures were collected from the hospital files and those of the Venereal Diseases Clinic.

Specimen collection. During the study period it was hospital policy to obtain specimens for culture of *N. gonorrhoeae* and *C. trachomatis* from all women presenting with abdominal pain. For the culture of *N. gonorrhoeae*, specimens were obtained from the urethra, the endocervical canal, and the cryptal area of the anus.

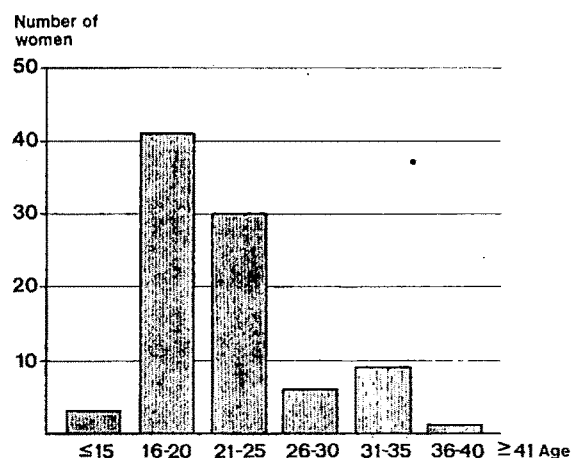


Fig. 2. Age distribution of women with acute salpingitis showing positive cultures for *N. gonorrhoeae*, *C. trachomatis*, or both (n = 90).

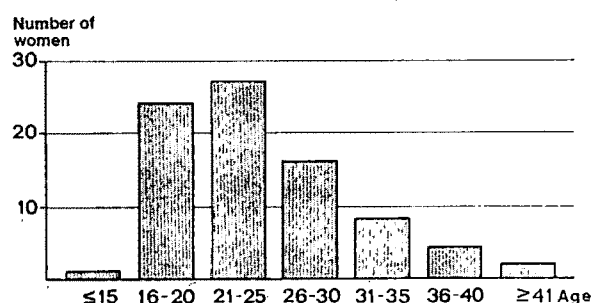


Fig. 3. Age distribution of women with acute salpingitis showing negative cultures for *N. gonorrhoeae* and *C. trachomatis* and not receiving antibiotics on admission (n = 82).

Specimens for the culture of *C. trachomatis* were obtained from the endocervical canal. Urethral swabs were collected from all sexual partners examined at the Venereal Diseases Clinic and cultured for *N. gonorrhoeae* and *C. trachomatis*. Microbiologic tests were performed at the Division of Bacteriology, Department of Pathology, University of Iceland, and evaluated by one of us (O. S.).

Microbiology. For the culture of *N. gonorrhoeae*, all specimens were collected with Culturette collection kits (Marion Scientific, Kansas City, MO 64114) and transported to the laboratory within 3 hours. Specimens were streaked on Thayer-Martin plates and incubated at 36° C for 48 hours in 5% carbon dioxide. *N. gonorrhoeae* was presumptively identified by colony morphologic features, microscopy, and oxidase tests. Identification was confirmed by the direct fluorescent antibody test.

All specimens from men were prepared with Gram's stain. If intracellular gram-negative diplococci were seen, the individual was considered to have gonorrhea, even if culture was negative.

Table I. Microbiologic culture results in women with acute salpingitis by marital state (n = 225)

Results of culture for gonorrhea and/or Chlamydia	Total	Cohabiting		Single	
		No.	%	No.	%
Positive	90	25	27.8	65	72.2
Negative	82	43	52.4	39	47.6
No culture or receiving antibiotics	53	30	56.6	23	43.4

Table II. Grading of salpingitis in relation to bacteriologic results

Results of culture	n	Mild (No.)	Moderate (No.)	Severe (No.)
Negative	82	37	29	16
Gonorrhea positive	25	9	14	2
Chlamydia positive	56	19	27	10
Gonorrhea and Chla- mydia positive	9	1	4	4
No culture or receiving antibiotics	53	25	13	15
Total	225	91	87	47

For the culture of *C. trachomatis*, specimens were collected on cotton-tipped swabs (Medical Wire and Equipment Co., Ltd., Corsham, Wilts., England) and sent within 3 hours in transport medium to the laboratory, where they were kept frozen at -70°C until cultured. The transport medium was 0.2 mol/L sucrose phosphate buffer with 10% fetal calf serum, 20 $\mu\text{g}/\text{ml}$ of gentamicin, 25 $\mu\text{g}/\text{ml}$ of vancomycin, and 2.5 $\mu\text{g}/\text{ml}$ of amphotericin. Tissue culture was performed with McCoy cells as follows: A monolayer was grown in CMA (complete medium with antibiotics) consisting of RPMI 1640 together with 20 mmol/L Hepes buffer, 1.3 mg/ml of gentamicin, 25 $\mu\text{g}/\text{ml}$ of vancomycin, and 2.5 $\mu\text{g}/\text{ml}$ of amphotericin. After approximately 20 hours, the medium was changed and CMA with 5% glucose substituted. The specimen was then centrifuged onto the monolayer at more than $5000 \times g$ and the temperature kept at 37°C . After 2 hours in an incubator, the medium was again changed and CMA with 5% glucose and 2 $\mu\text{g}/\text{ml}$ of cycloheximide substituted. After 72 hours of incubation at 36°C , the monolayer was fixed in methanol and stained with iodine. All specimens were cultured on two slides. If the results were inconclusive on examination of the first slide, the monolayer on the second slide was subcultured. If the first slide was distinctly positive or negative, the second one was also stained and examined. When the culture was inconclusive because of the cytopathogenic effect of the specimen, an attempt was made to obtain another specimen if the patient was not receiving antibiotics.

Statistical evaluation. The χ^2 test with Yates' correction was used for statistical analysis.

Results

Bacteriology. In the study population (n = 225), culture for *N. gonorrhoeae* was carried out in 214 women and for *C. trachomatis* in 207. In 11 cases, no record of culture was found. Thirty-four women were receiving antibiotic therapy on admission. Cultures for both organisms were obtained in 33 of these and for *N. gonorrhoeae* alone in one. All cultures from women receiving antibiotics were negative.

Culture for *N. gonorrhoeae* was done in 180 women not receiving antibiotics on admission. Thirty-four of these had positive cultures (18.9%).

Culture for *C. trachomatis* was done in 174 women not receiving antibiotics on admission. A cytopathogenic effect was present in five but repeat cultures were not done. In the remaining 169, there were 65 (38.5%) with positive cultures.

In all, 90 women had positive cultures. Eighty-one had positive cultures for one organism and nine for both.

Age and marital status. Fig. 1 shows the age distribution of the whole study population. Salpingitis was mainly confined to young women (mean 24.7 years, range 15 to 45). The age distribution of women with microbiologic evidence of sexually transmitted disease (mean 22.1 years, range 15 to 38) is shown in Fig. 2 and contrasts with that of women with negative cultures (Fig. 3) (mean 24.8 years, range 15 to 43). The incidence of positive and negative cultures was compared in two age groups, those 25 years of age or younger and those 26 years of age or older. The women with positive cultures were found significantly more often in the younger age group than in the older one

Table III. Sexual partners of women with culture-positive salpingitis: Relation to marital state and results of culture

Marital state	Sexual partners				
	n	Range per woman (No.)	Mean per woman (No.)	Cultured	
				No.	%
Cohabiting with gonorrhea (n = 7)	11	1-2	1.57	4	36
Single with gonorrhea (n = 18)	32	1-5	1.78	13	56
Cohabiting with <i>Chlamydia</i> (n = 18)	20	1-2	1.11	15	75
Single with <i>Chlamydia</i> (n = 38)	71	1-9	1.86	42	59
Single with gonorrhea and <i>Chlamydia</i> (n = 9)	21	1-4	2.33	14	67
Total	155			93	

Table IV. Results of cultures in sexual partners of women with salpingitis and positive cultures

Marital state	Cultures in sexual partners								
	No. of cultures from sexual partners	Gonorrhea positive		Chlamydia positive		Gonorrhea and Chlamydia positive		Negative	
		No.	%	No.	%	No.	%	No.	%
Cohabiting with gonorrhea (n = 7)	4	2	50.0	0		0		2	50.0
Single with gonorrhea (n = 18)	18	4	22.2	1	5.5	2	11.1	11	61.1
Cohabiting with Chlamydia (n = 18)	15	0		7	46.7	1	6.7	7	46.7
Single with Chlamydia (n = 38)	42	0		19	45.2	1	2.3	22	52.4
Single with gonorrhea and Chlamydia (n = 9)	14	3	21.4	2	14.2	3	21.4	6	42.9
Total	93	9	9.7	29	31.2	7	7.5	48	51.6

($\chi^2 = 6.86$, $p < 0.01$). There was no significant difference in age distribution among women with negative cultures.

Of the study population (n = 225), 127 (56.4%) were single (not cohabiting) and 98 (43.6%) were cohabiting (married or unmarried). Table I shows that women with positive cultures for one or both organisms were more often single (72.2%) than those with negative cultures (47.6%). This difference was significant ($\chi^2 = 9.91$, $p < 0.01$).

Analysis of the 90 women with positive cultures showed that 18 (72%) of the 25 women with gonorrhea alone were single. Of the 56 women with cervical *Chlamydia* alone, 38 (68%) were single. All nine women with both infections were single.

Severity of disease. The relation between severity grading of tubal inflammation found at laparoscopy or laparotomy and results of culture is shown in Table II.

Of the 82 women with negative cultures, 37 were considered to have mild and 45 to have moderate or severe salpingitis. In the 90 women with positive cultures, these figures were 29 and 61, respectively.

Although there was a trend toward more severe inflammatory changes in women with positive cultures compared with those in whom culture was negative, the difference between the groups was not significant ($\chi^2 = 2.50$, $0.1 < p < 0.5$).

Sexual partners. In the group of women with positive cultures (90) the number of sexual partners ranged from 1 to 9 with a mean of 1.7. The relation between marital state and the number of partners is shown in Table III. The 25 cohabiting women reported 31 sexual partners (range 1 to 2, mean 1.2), but the 65 single ones had 124 partners (range 1 to 9, mean 1.9). The number of partners was similar among women with gonorrhea alone (range 1 to 5, mean 1.7) and those

with *Chlamydia* alone (range 1 to 9, mean 1.6). The nine women infected by both organisms had the highest number of sexual partners (range 1 to 4, mean 2.3). Of the whole group, 56 (62%) reported only one sexual partner during the preceding 6 months.

Women with *Chlamydia* had only one partner (2.5:1 ratio of single/multiple partners) significantly more often than those with gonorrhea (ratio of single/multiple partners 1:1) ($\chi^2 = 4.36$, $p < 0.05$).

Of the 155 partners, 62 (40%) did not attend the Venereal Diseases Clinic. The reasons given were that the woman was unable to give accurate information about her partner's whereabouts or that the partner denied sexual contact, refused to be examined, lived abroad or elsewhere in Iceland, or wished to be examined and treated elsewhere. Ten of the men (6.5%) were foreign.

Urethral swabs for the culture of both organisms were obtained from 93 of the partners (60%) (Table IV), of which 45 (48.4%) were positive. Twenty percent had gonorrhea alone, 64.5% had *Chlamydia* alone, while 15.5% had both. There was a high incidence of positive *Chlamydia* cultures where such cultures had been positive in the woman.

Comment

In this study the clinical diagnosis of salpingitis was verified by laparoscopy or laparotomy during the acute phase of the disease in all cases. The importance of visual verification in suspected salpingitis was demonstrated by Jacobson and Weström,⁷ who found that in 35% of their cases there was no evidence of pelvic inflammation. At our department the clinical diagnosis of acute salpingitis was confirmed by laparoscopy in only 71.2% of the patients in a previous study.⁸ Wölner-Hanssen et al.¹⁰ found evidence of acute salpingitis at laparoscopy in 73% of women who had positive cervical cultures for *Chlamydia* and symptoms suggestive of salpingitis, but in 27% the tubes were apparently normal. It has been suggested that endosalpingeal inflammation may be present even when overt laparoscopic signs of salpingitis are lacking but purulent discharge on the mucosal surface of the fimbriated end of the tube accompanies endosalpingitis and was one of the prerequisites for the laparoscopic diagnosis of salpingitis by the criteria used here.⁶

With reliable culture techniques, it was possible to demonstrate that half of the women with acute salpingitis were infected by either *N. gonorrhoeae* or *C. trachomatis* and a few by both. *Chlamydia* was twice as common as *N. gonorrhoeae*. A similar observation was made by Svensson et al.⁹ and by Gjønnaess et al.,¹ who found that cervical gonorrhea was much less common than cervical *Chlamydia* infection in their Norwegian study population. A decrease in the incidence of gonococcal

salpingitis has been observed in Sweden,⁴ and at our department it decreased from 28% in 1978 to 1980⁸ to 19% in the present study, in line with the general trend for the whole country. Several studies from North America and Scandinavia have demonstrated that women attending gynecologic outpatient clinics have a higher prevalence of chlamydial infection (6.6% to 22.0%) than gonorrhea (0.8% to 10%).¹²⁻¹⁴

Cervical *Chlamydia* has been shown to be present in 4% to 6% of healthy women.^{15, 16} It is not known whether chlamydial infection of the female genital tract is self-limiting, but Johannisson et al.¹⁷ found persistence of *Chlamydia* in half of untreated cases for more than 4 weeks. McCormack et al.¹⁶ reported that seven of 14 women with untreated chlamydial infection remained infected 17 months after the initial examination and some of them had not had sexual contacts between examinations. Preliminary results from an ongoing study in our population suggest that 4% of women screened at an antenatal clinic have cervical *Chlamydia*, while among women seeking termination of pregnancy the figure is nearly 14%. No figures on the prevalence of the organism in the Icelandic male population are available, except for men who attend the Venereal Diseases Clinic, 42% of whom harbor the organism.

In this study *Chlamydia trachomatis* was isolated from the cervical canal of 38.7% of women with salpingitis. Isolation rates of between 22% and 40% for women with salpingitis¹⁸ have previously been reported from Scandinavia. This could be an underestimate, since *Chlamydia* may remain undetected in 15% to 25% of cases if urethral samples are not taken.^{15, 17} Endometrial or tubal sampling may further enhance the isolation rate¹⁸ but such samples were not obtained as part of this study.

Our findings on age distribution confirm earlier work indicating that acute salpingitis is primarily a disease of young women^{6, 9} and that there is a significant tendency for women with positive cultures to be younger than those with negative cultures.^{1, 3, 19} Differences in sexual life-styles, contraceptive practices or age-related differences in host-defense mechanisms could be possible explanations. Of the whole study population just over half were single. Women with positive cultures were single significantly more often than those with negative cultures. The highest number of positive cultures was thus found among young single women. The ratio of single/cohabiting women was, however, the same whether the woman had chlamydial or gonorrheal infection. The marital state and sexual life-style of the Icelandic population are probably similar to those of other Scandinavian peoples. Cohabitation without marriage is common. This cannot be equated with sexual freedom. The geographic position of the country renders the population relatively static and few of the "at

risk" women we studied had foreign sexual contacts. The majority of the women had only one partner during the preceding 6-month period, which suggests that transmission of gonorrhea and particularly chlamydial infection in this population is more likely to occur from man to woman, rather than in the reverse direction. Adler et al.⁵ found no difference in the sexual habits of women with salpingitis compared with those of individually matched control women attending the same hospitals for family planning advice. More detailed information on the role of the man in transmitting *Chlamydia* is needed.

The trend toward more severe inflammatory change in women with positive cultures for *Chlamydia* or gonorrhea has been described previously.^{1, 3, 18, 20} Gibson et al.²⁰ found that women with serologic evidence of past chlamydial infection were more likely to exhibit severe pelvic adhesions and hydrosalpinx formation, and similar findings have been described by others.^{21, 22} Svensson et al.⁵ found decreased fertility in patients with salpingitis compared with a control group, and women with negative cultures and those with gonorrhea alone had significantly milder inflammatory tubal changes than women with *Chlamydia*. The clinical symptoms however, are milder in salpingitis associated with *Chlamydia* infection.^{12, 18}

It has been shown that nearly one in five women aged 25 to 34 years became infertile after one episode of laparoscopically diagnosed salpingitis, and 31% and 60%, respectively, were infertile after second and third episodes.⁴ When salpingitis associated with a sexually transmitted disease is diagnosed, it is vital to explain the situation fully to the patient and to examine and treat her sexual partner or partners where possible to prevent reinfection and repeat salpingitis. In this study 60% of the men came for examination. About 50% of the men were found to be infected and received treatment, but a considerable number of potentially infected men did not receive treatment. To achieve a decrease in the incidence of repeat salpingitis, better contact tracing combined with accurate microbiologic diagnosis is necessary. The practice of giving antibiotics to women who may have salpingitis "to be on the safe side" without prior bacteriologic investigation is widespread but poor gynecologic procedure. Perhaps the time has come to examine microbiologically the partners of women with salpingitis on a routine basis as an important preventative measure, irrespective of the results of culture for sexually transmitted disease in the woman herself.

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The fetal-pelvic index as an indicator of fetal-pelvic disproportion: A preliminary report

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The objective of this study was to develop a standardized method of identifying fetal-pelvic disproportion by comparing fetal head and abdomen circumferences with the respective maternal pelvic inlet and midpelvis circumferences. Fetal ultrasound and maternal x-ray pelvimetry measurements were performed on 75 term pregnant women with indications for a trial of labor. By means of individually measured fetal and maternal pelvic diameters, circumferences of the fetal head, fetal abdomen, maternal pelvic inlet, and maternal midpelvis were computed. Based on four circumference differences between the fetus and maternal pelvis (fetal head — maternal pelvic inlet, fetal head — maternal midpelvis, fetal abdomen — maternal pelvic inlet, and fetal abdomen — maternal midpelvis), a fetal pelvic index number was derived from the sum of the two most positive values. Of the 27 patients requiring cesarean sections for failure to progress in labor, all but four had positive fetal pelvic index values (sensitivity = 85%). Of the 48 patients who were delivered vaginally, all but four had negative fetal pelvic index values (specificity = 92%). (AM J OBSTET GYNECOL 1986;155:608-13.)

Key words: Fetal-pelvic index, fetal-pelvic disproportion, x-ray pelvimetry, cephalopelvic disproportion, fetal ultrasound measurements

During the last four decades, various methods that address fetal-pelvic disproportion have been devised.¹⁻⁶ These include a clinical estimation of both pelvic size and fetal weight²; x-ray pelvimetry mensuration³⁻⁵ (that is, Colcher-Sussman technique, Mengert's index, Allen's areas, Moir's approach, and Weinberg and Scadron's sum); ultrasound assessment of fetal weight⁶; or a trial of labor for all fetuses in cephalic presentations.⁵ Although each of these methods has demonstrated limited success, none has proved reliable in prospectively identifying fetal-pelvic disproportion.^{1, 5, 7} As a result, many investigators believe an accurate diagnosis of fetal-pelvic disproportion can be made only in retrospect.^{1, 8} Conversely, to avoid any potential maternal or fetal sequelae of prolonged and unsuccessful labor trials, a means of prospective recognition of absolute and relative fetal-pelvic disproportion would be helpful.

The objective of this study is to develop a standardized method of identifying fetal-pelvic disproportion

by comparing fetal head and abdomen circumferences with respective maternal pelvic inlet and midpelvis circumferences. The accuracy of this method was tested in a blinded manner and compared with that of three other current methods of identifying fetal pelvic disproportion.

Methods

Seventy-nine pregnant women, at term gestation with indications for a trial of labor, were randomly entered into the study. Four patients were excluded from data analysis. Each of these patients underwent a cesarean delivery because of fetal distress that occurred before an adequate labor trial. Of the 75 study subjects in whom adequate labor trials were observed, 23 demonstrated spontaneous labor, 23 required oxytocin for induction of labor (for postdate pregnancy, diabetes mellitus, or ruptured membranes), and 29 required oxytocin augmentations during labor because of arrested labor patterns. All 75 fetal presentations were cephalic.

Ultrasound measurements of the fetal head and abdomen as well as maternal x-ray pelvimetry were performed before either the onset of active labor or oxytocin administration for the following reasons: (1) in the 23 patients with spontaneous labor, because fetal-pelvic disproportion was clinically suspected (large fetus or borderline-contracted maternal pelvis) and (2) in the other 52 patients, because the administration of intravenous oxytocin was necessary to induce or augment labor.

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Table I. Results of descriptive variables

Outcome	n	Age (yr)	Parity	Gestational age (wk)	Type of labor				Actual birth weight
					Spontaneous	Oxytocin			
						Induction	Augmentation	Maximum dose (mU/min)	
Cesarean	27	22 ± 4	0.7 ± 1	40 ± 2	8	7	12	9 ± 8	3708 ± 482
Vaginal	48	22 ± 4	0.9 ± 1	40 ± 2	15	16	17	6 ± 6	3546 ± 604

Fetal ultrasound evaluations were performed in the labor rooms with use of a portable ADR 4000 S/L linear-array scanner with a 3.5 MHz transducer calibrated to a sound velocity in soft tissue of 1540 m/sec (Advanced Technology Labs Inc., Tempe, Arizona). Diameters of the fetal head—outer-to-inner biparietal, outer-to-outer transverse (TD_h), and outer-to-outer anterior-posterior (APD_h)—were determined by means of built-in electronic calipers applied to a freeze-frame image of the plane that included the thalamus, the septum cavum pellucidum, and a symmetrically aligned midline echo. Fetal abdominal diameters were also determined from a freeze-frame image that visualized the plane perpendicular to the fetal spine at the level of the portal vein bifurcation and stomach. By means of electronic calipers, the anterior-posterior diameter (APD_a) was measured from the outer aspect of the fetal spine to the outer anterior abdominal wall. The outer-to-outer transverse diameter (TD_a) was measured 90 degrees to the APD_a .

With regard to the maternal pelvic measurements, respective anterior-posterior and transverse diameters of the inlet (APD_i and TD_i) and midpelvis (APD_m and TD_m) were measured by means of x-ray pelvimetry (Colcher-Sussman technique).⁴

From the four sites of individually measured diameters (fetal head and abdomen, and maternal pelvic inlet and midpelvis), respective circumferences of the fetal head (HC), fetal abdomen (AC), maternal pelvic inlet (IC) and maternal midpelvis (MC) were computed with use of the following simple equation: $C = (TD + APD) \times \frac{1}{2}\pi$. Based on the four circumference differences between the fetus and maternal pelvis (cephalopelvis differences: $HC - IC$ and $HC - MC$; abdominopelvis: $AC - IC$ and $AC - MC$), a fetal-pelvic index number was derived from the sum of the two most positive circumference differences (+ number = presence of fetal-pelvic disproportion; - number = absence of fetal-pelvic disproportion). Equations for the fetal ultrasound evaluations were $HC = (TD_h + APD_h) \times 1.57$ and $AC = (TD_a + APD_a) \times 1.57$. Equations for the x-ray pelvimetry were $IC = (TD_i + APD_i) \times 1.57$ and $MC = (TD_m + APD_m) \times 1.57$. For comparative data, three other methods of identifying

ing fetal-pelvic disproportion were analyzed. These included ultrasound-derived estimated fetal weights (Shepard's tables)⁶ and two x-ray pelvimetry techniques (Colcher-Sussman⁴ and Mengert's index⁹).

Results of the fetal-pelvic index were blinded from the clinicians managing the patient's labor, and outcome results were blinded from the investigators. In all 75 patients, adequate labor trials were observed and defined as (1) uterine contractions of at least 50 mm Hg in intensity and 60 seconds in duration, (2) a frequency of at least three contractions in 10 minutes, and (3) cervical dilation to ≥ 5 cm for a minimum of 2 hours. Cesarean sections were performed on those patients in whom the lack of progress of labor was observed, and fetal-pelvic disproportion was clinically determined. This study was reviewed and approved by the investigational review board. These data were analyzed by means of the Fisher's exact and *t* tests with use of the STAT PAK program on a Hewlett-Packard 150 computer. Results of the four methods were compared with regard to delivery outcome.

Results

Descriptive variables of sample population. Descriptive variables for the two study groups are given in Table I. Following an adequate trial of labor, 27 patients (36%) required cesarean sections and 48 patients (64%) were delivered vaginally. Of the 27 patients requiring cesarean delivery, eight demonstrated spontaneous labor, seven received intravenous oxytocin inductions of labor, and 12 were given intravenous oxytocin augmentations. Of the 48 patients with vaginal deliveries, 15 demonstrated spontaneous labor, 16 received intravenous oxytocin inductions, and 17 were given intravenous oxytocin augmentations. The mean maximum dose of oxytocin between the two groups was not significantly different. The mean actual birth weight $\pm$ SD was 3708 ± 482 gm for the cesarean group and 3546 ± 604 gm for the vaginal delivery group.

Estimated fetal weight and x-ray pelvimetry techniques. Results of the study data are given in Table II. Estimated fetal weights were computed from ultrasound-measured fetal biparietal diameters and abdom-

Table II. Study data results* (n = 75)

Outcome	Ultrasound—EFW (gm)		X-ray pelvimetry				FPI	
	≥4100	<4100	Colcher-Sussman		Mengert's index		+	−
			Contracted	Adequate	Contracted	Adequate		
Cesarean	5	22	5	22	7	20	23	4
Vaginal	7	41	0	48	2	46	4	44
Sensitivity (%)	19		19		26		85	
Specificity (%)	85		100		96		92	
Overall predictability (%)	61		71		71		89	
p Value*	NS		<0.005		<0.01		<0.001	

EFW = Estimated fetal weight; FPI = fetal pelvic index.

*Fisher's exact test.

Table III. Results of the measurement and fetal-pelvic index data*

Study group	n	ABW	Ultrasound		X-ray pelvimetry		FPI
			HC	AC	IC	MC	
Cesarean section	27	3708 ± 482	33.4 ± 1.1	35.7 ± 2.4	38.7 ± 2.2	33.8 ± 1.5	+2.1 ± 3.6
Vaginal delivery	48	3546 ± 605	33.1 ± 1.5	34.5 ± 2.3	40.4 ± 2.5	36.1 ± 2.3	−4.4 ± 4.6
t Test		1.194	0.910	2.132	2.945	4.663	5.110
p Value		NS	NS	<0.05	<0.01	<0.001	<0.001

*Mean ± standard deviation.

inal circumferences by means of the Shepard tables.⁶ When an estimated fetal weight of ≥4100 gm was used as the threshold weight for identifying the presence of fetal-pelvic disproportion, the sensitivity was 19%, the specificity was 85%, and the overall predictability was 61%. When the Colcher-Sussman technique was used alone (a contracted pelvis is defined as <22 cm for the inlet or <20 cm for the midpelvis), the sensitivity was 19%, the specificity was 100%, and the overall predictability was 71%. Using Mengert's index alone in which a contracted pelvis is defined as <85% of average pelvic capacity (<123 cm² for the inlet or <106 cm² for the midpelvis), the sensitivity was 26%, the specificity was 96%, and the overall predictability was 71%.

Analysis of these data demonstrates that ultrasound-derived estimated fetal weights and x-ray pelvimetry techniques used alone are imprecise in identifying the presence of fetal-pelvic disproportion as a result of their low sensitivities.

Fetal-pelvic index. Results of the measurement and fetal-pelvic index data are given in Table III. When compared with those of the cesarean section group, actual birth weights as well as fetal head circumferences for the vaginal delivery group were not significantly different. However, significant differences between the two groups are reported with regard to the fetal abdominal, maternal pelvic inlet, and maternal midpelvic circumferences. The most significant difference be-

tween the two groups was observed in the fetal-pelvic index.

When compared with the other methods tested, the ability to identify the presence or absence of fetal-pelvic disproportion is markedly improved when the fetal-pelvic index method is used (Table II). Of the 27 patients requiring cesarean deliveries for lack of progress in labor, 23 demonstrated positive fetal-pelvic indices, and four had negative fetal-pelvic indices (sensitivity, 85%). Although these four patients with negative fetal-pelvic indices represent false negatives, each fetus presented in an occiput posterior position and failed to progress in labor despite adequate contractions.

Of the 48 patients who delivered vaginally, 44 demonstrated negative fetal-pelvic indices and four had positive fetal-pelvic indices (specificity 92%). Although these four patients with positive fetal-pelvic indices delivered vaginally, in retrospect, each demonstrated a degree of fetal-pelvic disproportion that resulted in a difficult vaginal delivery. The first patient demonstrated a prolonged second stage of labor and was delivered of a 3100 gm infant following a difficult midforceps rotation from a persistent occiput transverse position at a +2 station. The second patient underwent a difficult midforceps delivery of a 4000 gm infant. The third patient was delivered of a 4580 gm infant in whom suprapubic pressure and a Woods' maneuver were required to reduce a shoulder dystocia. The fourth pa-

tient was delivered of a 4050 gm infant by vacuum extractor, also from the midpelvis, following numerous applications.

Based on these preliminary data, the fetal-pelvic index is an accurate method of identifying the presence or absence of fetal-pelvic disproportion with an overall delivery outcome predictability of 89% (Fig. 1).

Comment

Historically, obstetricians have observed high maternal and perinatal morbidity and mortality rates for patients undergoing cesarean deliveries following prolonged labors.^{9,10} Despite modern techniques of intrapartum fetal monitoring, many investigators have sought a method that will identify fetal-pelvic disproportion with use of the onset of labor.^{1-5,7,11}

X-ray pelvimetry. Before 1944,⁴ numerous x-ray mensuration techniques such as those of Jarcho, Thoms, Caldwell and Moloy, Snow, Weitzner, and others were described. Each of these methods provided limited predictability of fetal-pelvic disproportion, but no one method was universally adopted.

All of the above methods failed to use intersecting diameters of the true pelvis, simple positioning of the patient, and a ruler for straightforward mensurations. The method that first fulfilled these basic requirements was described in 1946 by Colcher and Sussman.⁴ Based on a sum of the transverse and anterior-posterior diameters of the pelvic inlet as well as that of the midpelvis, they defined an adequate pelvic size as being >22 cm for the inlet and >20 cm for the midpelvis.

In 1948 Mengert³ described five components of cephalopelvic disproportion: (1) size and shape of the bony pelvis, (2) size of the fetal head, (3) force exerted by the uterus, (4) moldability of the fetal head, and (5) fetal presentation and position. At that time, he believed that only the first of these components could be measured accurately. In an effort to more accurately estimate the capacity of the pelvis, Mengert used x-ray pelvimetry measurements and calculated products of the transverse and anterior-posterior diameters for both the inlet and midpelvis. Based on his data from 592 patients, average area values of 145 cm² for the inlet and 125 cm² for the midpelvis were computed. By a process of trial and error, he found that 85% of normal, for either the inlet (123 cm²) or midpelvis (106 cm²), represented the borderline between pelvic adequacy and contraction.

In 1951 Kaltreider⁵ reported his studies on the criteria for pelvic inlet contraction. Pelvic size estimates were usually made on the basis of the estimated true conjugate as determined by manual measurement of the obstetric conjugate. He concluded that (1) for all patients in whom there has not been a previous cesarean section and in whom there is a vertex presentation,

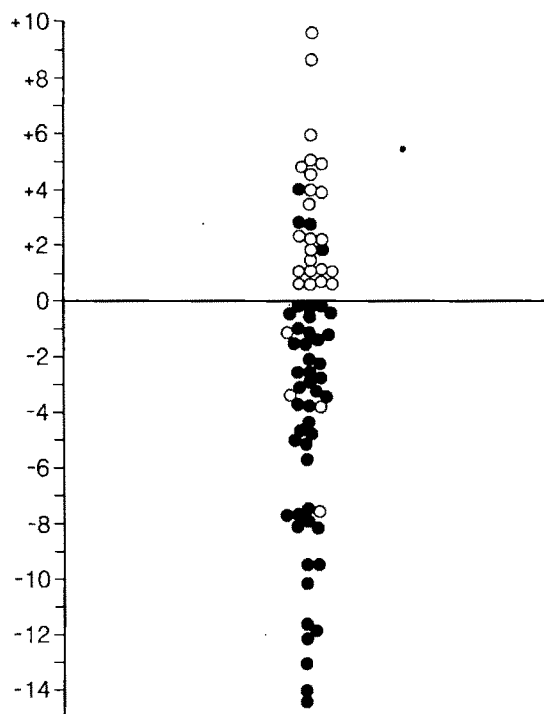


Fig. 1. Scattergram results of the fetal-pelvic index and delivery outcome. A positive (+) fetal pelvic index indicates the presence of fetal-pelvic disproportion; a negative (−) fetal pelvic index indicates the absence of fetal-pelvic disproportion. The open circles represent patients who were delivered by cesarean section and the closed circles represent those who were delivered vaginally ($p < 0.001$).

a trial of labor is indicated; (2) labor will decide whether vaginal delivery is possible or cesarean section necessary; (3) lateral pelvimetry during labor will prevent misinterpretation of pelvic findings when molding and caput are present; (4) uterine inertia in a patient with a borderline contracted pelvis should be delivered by cesarean section; (5) since the above methods cannot give an absolute prediction in abnormal presentations (for example, breech), obstetric and x-ray evaluation of the maternal pelvis together with clinical judgement must be used.

In the 1970s the value of x-ray pelvimetry for identifying fetal-pelvic disproportion was scrutinized and fell into disfavor because investigators considered it of little value as a prognostic tool in labor.^{1,2,5,7,8} Unfortunately, most of the x-ray pelvimetry studies were done without considering fetal size or the benefit of statistical analysis.^{1-5,7}

Joyce et al.⁷ and Jagani et al.¹ evaluated the usefulness of x-ray pelvimetry in predicting the outcome of labor and stated that it is limited by the difficulties of assessing fetal weight. However, they further reported that when actual birth weights were plotted as a function of the obstetric conjugate, a zone was found to exist in which

no vaginal delivery occurred and thus the failure of vaginal delivery could be predicted. They further stated that obstetricians unfortunately focus on the fetal head because it comes first. Based on their observations, they stated that the term "cephalopelvic disproportion" should be abandoned because fetal size and weight both correlate better with fetal abdominal dimensions than with those of the fetal head. Moreover, these investigators predicted that a prospective study with use of ultrasonography to estimate fetal size and radiography to estimate maternal pelvic size along with data analysis might be productive.^{1,7}

Results of our data demonstrate that by use of ultrasound fetal measurements together with respective maternal x-ray pelvic measurements, the presence or absence of fetal-pelvic disproportion and delivery outcome can be predicted with an accuracy of 89%.

Ultrasound. Following the advent of ultrasound, measurements of the fetal head and abdomen proved to be accurate parameters for estimating fetal weight. A number of equations for the purpose of estimating fetal weight, based on ultrasound measurements of the fetal biparietal diameter and abdominal circumference obtained within 48 to 72 hours before delivery, have been devised and tested.⁸

In our study we observed that many large fetuses were delivered vaginally without incident. Conversely, there were many average-sized fetuses for whom fetal-pelvic disproportion existed and vaginal delivery was not possible. Thus fetal weight is neither a good indicator of fetal-pelvic disproportion nor an accurate predictor of delivery outcome without consideration of maternal pelvic dimensions.

Trial of labor. A trial of labor for all cephalic presentations has been recommended by many investigators,^{1,5,7} but this approach may not be free of morbidity if fetal-pelvic disproportion is present.¹⁰ In women with labors of >20 hours or in those with a second stage of labor >3 hours, significantly increased perinatal mortality rates have been observed. In many pregnant women the degree of the fetal-pelvic disproportion becomes apparent only when one or more of the following occur: a persistent delay in cervical dilation, fetal heart rate changes, meconium in the amniotic fluid, or failure of descent of the fetal head with progressive molding.⁸⁻¹⁰ These signs are not always detected in time, although their early recognition may be critical to fetal well-being.

Based on the presence of a positive fetal-pelvic index, 27 study patients were identified as having fetal-pelvic disproportion (Table II). Of these patients, 23 demonstrated failure to progress in labor and required cesarean sections. The other four underwent difficult midpelvic operative vaginal deliveries. Based on the presence of a negative fetal-pelvic index, 48 study pa-

tients were identified as not having fetal-pelvic disproportion. Of these patients, four demonstrated failure to progress in labor and required cesarean sections. The other 44 were delivered vaginally without incident.

Side effects of x-ray pelvimetry and ultrasound. An important issue is the concern of possible cancer induction in those individuals exposed to third-trimester prenatal radiography. Throughout various geographic areas of the world, it is known that natural background radiation ranges from approximately 80 mrad to well over 1000 mrad per year. The average total exposure to the fetus over a 9-month period from natural sources is approximately 86 mrad.⁸ In comparison, total exposure to the fetus from x-ray pelvimetry is approximately 80 mrad.¹²

Collectively the data compiled from 15 investigators studying the incidence of leukemia in children exposed to radiation in utero show a relative risk for childhood cancer of approximately 1.40.⁸ Seven of the 15 studies demonstrated an increased relative risk to the fetus, and eight showed no increased risk. Unfortunately, most of the studies that showed an increased relative risk were based on retrospective data analysis,⁸ and the recent twin study does not provide an unequivocal answer.¹³ Furthermore, Japanese fetuses experiencing in utero exposure to radiation associated with atomic bombs have not demonstrated an increased cancer risk.¹⁴ Convincing evidence of an increased relative risk of childhood cancer from third-trimester, in utero, low-dose irradiation is lacking.^{8,13}

With regard to diagnostic ultrasound, there is no conclusive evidence that supports any hazard to the fetus. In 1982 the following statements were issued by the American Institute of Ultrasound in Medicine as its official position on ultrasound safety¹⁵:

1) Given the known benefits and recognized efficacy of the use of ultrasound for medical diagnosis, including use during human pregnancy, the American Institute of Ultrasound in Medicine herein addresses the clinical safety of such use. 2) Diagnostic ultrasound has been in use for over 25 years. No adverse biological effects on patients or instrument operators caused by exposures at intensities typical of present diagnostic instruments have ever been reported. Although the possibility exists that such biological effects may be identified in the future, current data indicate that the benefits to patients of the prudent use of diagnostic ultrasound far outweigh any potential risk.

Although fetal diagnostic ultrasound and maternal x-ray pelvimetry should not be used indiscriminately, in certain cases we feel that the potential benefits of using them prospectively to identify fetal-pelvic disproportion outweigh the potential risks of intravenous oxytocin and prolonged labor trials.

Assessment of fetal-pelvic disproportion. Except

for the monograph by Steer,¹⁶ with use of the Ball technique of x-ray pelvimetry, the term cephalopelvic disproportion has not been clearly defined. Previous methods of assessing fetal-pelvic disproportion have been imprecise because they evaluate the maternal pelvic size without considering fetal measurements other than those of the fetal head. Furthermore, cephalopelvic disproportion refers only to the size of the fetal cranium as compared with that of the maternal pelvis, and it ignores the girth of the fetal abdomen and shoulders.

This study uses maternal x-ray pelvic measurements and fetal ultrasound measurements in concert. By comparing the fetal head and abdomen circumferences with the respective maternal pelvic inlet and midpelvis circumferences, we believe that various degrees of absolute and relative fetal-pelvic disproportion can be accurately identified. Thus, on an individual basis, the relative fit of the passenger with the passage is compared. When the circumference of fetal head or abdomen is larger than that of the maternal pelvic inlet or midpelvis, either cephalopelvic disproportion, abdominal pelvic disproportion, or both can be prospectively recognized.

Although many investigators^{1, 3, 5, 7} believe that individual diameters of the fetal head and abdomen as well as those of the maternal pelvis can change in labor, we found the respective circumferences to be highly predictive of both fetal-pelvic disproportion and delivery outcome.

Based on our preliminary data, the fetal-pelvic index appears to be an accurate method of identifying fetal-pelvic disproportion and thus the need for abdominal delivery. Although the number of cases reported is small, the preliminary data are highly significant. The large-scale evaluation of this technique is currently being undertaken.

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Hydatidiform mole: Cytogenetic marker analysis in twin gestation

Report of two cases

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A hydatidiform mole associated with a fetus proved to be the result of twin gestation. On microscopic examination of the placenta the case was classified as a partial hydatidiform mole. Chromosomal markers were, however, consistent with a normal conception and a mole of diploid androgenetic origin.

Chromosome analysis of a morphologic complete molar specimen yielded two cell lines, one consistent with a normal conception and one with diploid androgenesis. Twinning in molar specimens must therefore be considered, regardless of macroscopic appearance. The prenatal diagnosis of a coexisting fetus and molar placenta poses a real clinical problem; analyses must distinguish between a partial mole plus a triploid fetus and a normal fetus occurring with a partial or a complete mole. The distinction is important for decisions made during pregnancy and may be of prognostic significance after termination. The usefulness of chromosome marker analysis in distinguishing between the various origins is pointed out, and it is suggested that twin pregnancy with hydatidiform mole is more frequent than its description in the literature would suggest. (AM J OBSTET GYNECOL 1986;155:614-7.)

Key words: Hydatidiform mole, twin pregnancy, diploid androgenesis, chromosome markers

Hydatidiform mole may be accompanied by a normal fetus or one with malformations. Triploidy has been the most frequent observation in cases where fetal malformation existed and/or in which a single placenta consistent with a partial mole was found. Conceptions consisting of a fetus without malformations and a placenta with a separate molar mass were of diploid chromosomal content in the few cases thus far reported (Table I).¹⁻⁷ In such cases karyotyping alone is of limited value for the confirmation of twin pregnancy; additional analyses of chromosomal markers may, however, reveal the origin of the chromosome complements and rule out maternal cell contamination as the source of a 46,XX karyotype. Furthermore, the observation in an apparently homogeneous specimen of two distinct cell lines may be the only indication that a "vanishing

twin" once existed. It has been stressed that "Where a fetus is present it is important that every effort should be made to distinguish between a complete hydatidiform mole with twin fetuses and a partial hydatidiform mole, since the potential for progressive diseases may be much less with the latter."⁸

We report two cases of hydatidiform mole in which twin gestation was confirmed by analysis of combined QM- and AgNOR-chromosomal markers.

Material and methods

Material for investigation of genetic markers was obtained as part of a Danish study on hydatidiform moles initiated in October 1982.⁹ During the first year (January 1 to December 31, 1984), when the investigation was supported by gynecologic and obstetric departments throughout the country, 52 molar specimens were diagnosed by the centralized histopathologic revision of specimens sent from the local departments of pathology (Bent Mogensen, personal communication). Of 18 cases having their origin in diploid androgenesis, as demonstrated by the study of chromosomal polymorphisms, two cases showed a second cell line consistent with twinning.

Tissue culture studies were established and chromosome marker analysis performed as previously described.¹⁰ In case 1 there was no fetus or membranes. Molar tissue was set up for both short- and long-term

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Table I. Chromosome analysis and/or morphology indicating twin gestation

Author	Year	Case No.	Gestational age (wk)	Fetal/placental karyotype	Fetal anomalies	Molar karyotype	Morphology	Placentation
Blanc et al. ¹	1970	1	38	46,XY	NO	—	Môle hydatidiform	NP + SHM
Honda et al. ²	1980	1	15	46,XY	NO	46,XX	Complete mole	NP + SHM
Sauerbrei et al. ³	1980	2	20	46,XX	NO	—	Complete mole	NP + SHM
		4	22	46,XY;46,XY	NO	—	Complete mole	NP + NP + SHM
Block and Merrill ⁴	1982	6	15	46,XY	NO	46,XY	Hydatidiform	SDM
Fisher et al. ⁵	1982	1	16	46,XY	NO	46,XX	Complete mole	NP + SHM
Teng and Ballon ⁵	1984	1	15	46,XX	—	46,XX	Incomplete HM	NP + SHM
		2	—	46,XY	NO	46,XY	Incomplete HM	NP + SHM
		3	20	46,XY	NO	46,XY	Incomplete HM	NP + SHM
Crooij et al. ⁷	1985	1	27	46,XX	NO	—	Partial mole	SDM
Present report	1986	1	22	46,XY	No fetus	46,XX	Complete mole	SDM
		2	22	46,XX	NO	46,XX	Atypical mole	NP + SHM

NO, Not observed; NP + SHM, normal placenta and separate hydatidiform mole; SDM, single placenta with diffuse molar change; HM, hydatidiform mole.

Table II. Chromosomal markers

	Sex	3	13	14	15	21	22
Case 1							
Spouse	XY	ab	ab	ab	ab	ab	ab
Line I	XX	aa	bb	bb	bb	aa	aa
Patient	XX	cb	cb	cb	ac	ac	cd
Line II	XY	cb	bb	cb	ac	aa	ca
Case 2							
Spouse	—	—	—	—	—	—	—
Hydatidiform mole	XX	dd	ee	ee	ff	cc	ff
Patient	XX	cd	cc	cd	cd	cd	cd
Fetus	XX	cd	ec	ed	ed	cd	ed

Chromosomal heteromorphisms on QM-banded and AgNOR-stained preparations excluding maternal cell contamination are in italics; *a, b, c, d, e*, and *f* are symbols and do not represent specific heteromorphisms; *e* and *f* are symbols of molar and/or fetal chromosomal markers that are not observed on parental chromosomes.

cultures. In case 2 metaphases were obtained from both fetal fibroblasts and molar villi. Chromosome polymorphisms of fetal and molar tissues were compared to parental markers revealed by QM-banding (quinacrine mustard) and AgNOR-staining of metaphases from peripheral blood cultures. In case 2 it was not possible to obtain a blood sample from the spouse. Chromosome marker analyses were performed without knowledge of the clinical or morphologic presentation of the molar specimen.

Case reports

Case 1. M. H., a 21-year-old primigravid woman, was referred at 22 weeks' gestation because of vaginal bleeding. The pregnancy was in the first trimester and was complicated by nausea, vomiting, and vaginal spotting. The patient had not noticed fetal movements. Ultrasound investigation revealed several anechoic spaces consistent with presence of a molar placenta. Two liters of molar tissue, without evidence of an associated fetus, were evacuated by suction.

Metaphases harvested after short-term culture showed a 46,XY karyotype and markers consistent with a normal conception. The long-term culture of molar

tissue, however, had a 46,XX karyotype. All molar chromosome markers investigated were identical on homologous chromosomes (Table II); this finding, which rules out a karyotype established from maternal cells, is in accordance with a diploid androgenetic origin of a complete mole.

Histopathologic examination revealed avascular villi with pronounced hydropic swelling. Fetal elements were not observed. A moderate trophoblastic overgrowth was present, and a diagnosis of complete hydatidiform mole was made. However, the mole had an uncommon characteristic. Proliferating villous stroma formed nuts into the cystic centers of many of the hydatidiform villi.

Serum human chorionic gonadotropin titer before evacuation was 11,600 IU/L. The titers decreased to the limit of detection within 5 weeks.

Case 2. J. C., a 25-year-old patient, gravida 5, with a history of four uncomplicated pregnancies, presented at 17 weeks' gestation with vaginal bleeding preceded by spotting at 12 and 15 weeks of pregnancy. Ultrasound examination revealed a live fetus with a biparietal diameter corresponding to gestational age (3.5 cm). Transsonic spaces in the placenta were interpreted as signs of previous bleeding. The patient refused further

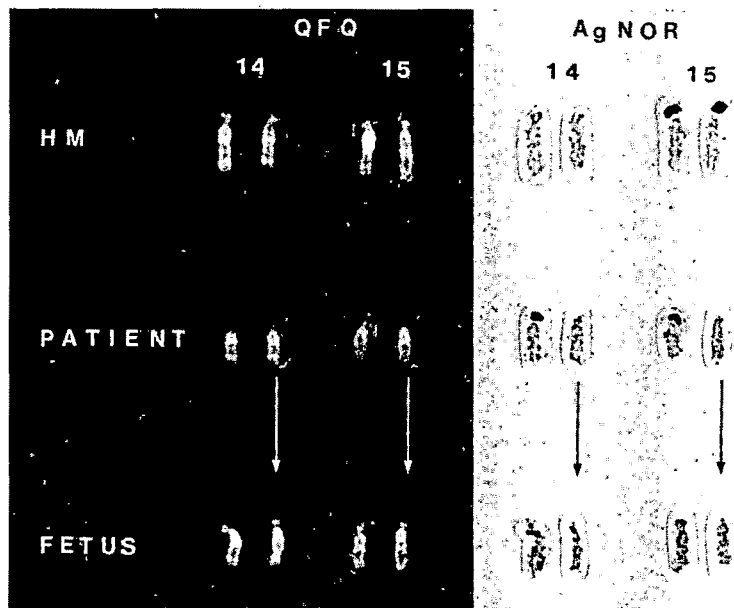


Fig. 1. QM-banded and AgNOR-stained partial karyotype excluding contaminating maternal cells. A paternal blood sample was not obtainable, but markers on molar chromosomes are consistent with diploid androgenesis (case 2).

examination until the twentieth week of gestation. Ultrasonographic examination at that time demonstrated a live fetus and an increased placental mass consistent with hydatidiform mole. Serum α -fetoprotein value was 290 KU/L, that is, two to three times the value expected at the actual time of gestation. It was not possible to aspirate amniotic fluid for chromosome analysis. The patient did not accept induced abortion but delivered at 22 weeks a dead female fetus tightly wrapped in the membranes, an apparently normal placenta, and a separate mass of molar tissue. No fetal malformations were revealed.

Metaphases obtained from cultured fetal fibroblasts showed a 46,XX karyotype as did metaphases on cultured molar tissue (Table II). Marker analysis on QM-banded and AgNOR-stained preparations revealed that homologous molar chromosomes numbers 13, 14, 15, and 22 were identical and that none of these markers were present in the patient (Fig. 1). Fetal markers indicated a maternal origin of one chromosome complement, whereas markers on the second chromosome of the pairs differed from those observed in metaphases from the patient. Maternal cell contamination as the source of the karyotypes in fetal and molar tissue were thus excluded. Chromosomal polymorphisms confirmed the diagnosis of a twin pregnancy, with a normal conception and a mole of diploid androgenetic origin.

Microscopic examination of the placenta demonstrated both normal and hydropic villi. The hydropic swelling was chiefly localized to the primary villi, and the trophoblastic growth appeared to be within normal range. The case was registered as a partial hydatidiform mole with an unusual appearance, the hydropic swelling being localized to the primary villi.

Serum human chorionic gonadotropin titer at evacuation was 81,000 IU/L. The level of serum human chorionic gonadotropin progressively declined after termination of the pregnancy to 200 IU/L within 8 weeks. The patient declined further examination.

Comment

Reports on hydatidiform mole in which a twin pregnancy was diagnosed or could not be excluded after chromosome analysis are listed in Table I. To our knowledge the only previously reported cases verified by chromosome polymorphisms as twin pregnancies were those described by Honda et al.² and Fisher et al.⁵ In contrast to the often malformed triploid fetuses found in association with partial, incomplete moles, no anomalies have been described in karyotypically diploid fetuses associated with hydatidiform mole (Table I). It is thus likely that these pregnancies include both a normal conceptus and a diploid mole. It remains to be elucidated whether the variable morphologic appearance of molar parts, as indicated by the two cases reported here, will be characterized by a common origin from diploid androgenesis. That would, however, be in accordance with the risk of malignant change evident from some cases.⁶

The observation of two twin gestations among 18 paternally derived diploid molar specimens contrasts to the low incidence previously assumed. This may indicate that the twinning rate in molar pregnancies is more likely to approach the incidence of approximately 1:30 which is calculated for spontaneous abortions in general.¹¹ The small number of cases reported could

be partly explained by the phenomenon of the vanishing twin; the number of pregnancies in which one conception is lost in utero is unknown. Estimates of disappearance rates range from 0% to 78%, depending on the population and the gestational age at which ultrasonographic studies were performed.¹² As demonstrated by case 1, some of the cases of twins in molar pregnancies are detectable only by investigation of genetic markers; it may be worthwhile to research such evidence of twin pregnancy even when the morphologic presentation is not suggestive of such.

Molar specimens have been classified as partial hydatidiform moles on the observation of fetal parts and/or membranes,¹³ or the chromosomal constitution has been assumed on the basis of morphology alone.¹⁴ Since investigation of genetic markers is essential for the distinction between partial mole and complete mole plus a fetus, appropriate cytogenetic studies should be attempted when the presence of hydatidiform mole with an associated fetus is diagnosed. Recent improvements in ultrasonographic scanners and increasing use of ultrasonography during the first trimester are likely to increase the number of observations of hydatidiform mole with associated fetus. This raises a difficult situation in the counseling of the patient, since the management of a pregnancy comprising a triploid fetus and a partial mole compared to that of a normal fetus accompanied by a complete mole may be quite different. The final diagnosis requires karyotyping and analysis of chromosomal markers, preferentially following amniocentesis. When fetal triploidy is observed or if the amount of amniotic fluid is reduced to such an extent that a fetus papyraceus is probable, termination of the pregnancy is advisable. However, when a normal diploid fetal karyotype is revealed, further management of the pregnancy must include other information as well: for a couple who conceives readily termination may be preferable to continuing a complicated gestation. If the pregnancy is "extremely precious," however, it is possible to continue, providing that careful monitoring is assured.

Extended application of cytogenetic investigations, including analysis of chromosomal polymorphic mark-

ers, appears to be of comprehensive clinical importance when applied prenatally or after abortion in these pregnancies. Further cases examined cytogenetically and histopathologically are necessary to clarify whether the characterization of an additional subgroup of "diploid partial moles" is justified.

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von Willebrand's disease and pregnancy: Management during delivery and outcome of offspring

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Six patients having different subtypes of von Willebrand's disease were followed up during eight complete pregnancies. Two additional pregnancies terminated in spontaneous abortions. Five pregnancies ended in cesarean section either because of obstetric problems (three) or electively (two) to avoid infant bleeding. Three deliveries were complicated by vaginal bleeding attributed to von Willebrand's disease, while bleeding during two deliveries had clear obstetric causes. Only two deliveries were associated with no bleeding complications. Five newborn babies were found to have von Willebrand's disease. One of them was born with a head hematoma. Management, which included cryoprecipitate and desmopressin (Stimate), is discussed. It is important to manage each case individually since obstetric parameters and severity of bleeding disorder must be known before treatment is planned. (*AM J OBSTET GYNECOL* 1986;155:618-24.)

Key words: von Willebrand's disease, pregnancy

von Willebrand's disease is a hemorrhagic disorder characterized by the appearance of spontaneous mucocutaneous hematomas, easy bruisability, epistaxis, and in severe cases profuse menstrual bleeding, menorrhagia, and intra-articular bleeding. In most families this disorder is inherited as an autosomal dominant condition; however, there are some reports of other patterns of inheritance and in rare instances the disorder can be acquired.¹ All families known to us with this condition are offered genetic counseling and family planning help. In many cases, because of the relative mildness of the disorders, the family elects to have children, and in such cases the pregnancy may be taxing to mother, obstetrician, and hematologist. The main laboratory findings include a prolonged bleeding time, decreased platelet retention in glass bead columns, abnormal ristocetin-induced platelet agglutination, and variable quantitative and/or qualitative abnormalities of components of the factor VIII complex.

It has been widely accepted that the factor VIII complex is composed of two recognizable glycoproteins that are controlled by genes on different chromosomes. The largest, factor VIII-related antigen (VIII R:Ag, which is coming to be called the von Willebrand factor antigen or VWF:Ag) is inherited under the influence of an autosomal gene and it is produced by endothelial cells. The other one, much smaller, is factor VIII coagulant

(VIII:C), produced under the influence of a gene localized in the X chromosome and possibly by a cell in the liver. The release of factor VWF:Ag from endothelial cells induces the production and/or the release of factor VIII:C and then the two proteins link to circulate as a complex. These two glycoproteins have distinct functional expressions. Factor VIII:C, which is deficient in patients with hemophilia A and some carriers of this disorder, participates in clot formation and is usually measured by the partial thromboplastin time or activated partial thromboplastin time or by the one- or two-stage factor VIII:C analysis. The functional expression of the other glycoprotein, von Willebrand factor, can be assessed either by the Weiss assay or ristocetin cofactor activity (RiCof)² or by direct agglutination of the patient's platelets in response to ristocetin. Finally, the bleeding time, which is an overall expression of the relationship of platelets with subendothelium, serves as a check on laboratory assessment since VWF:Ag is one of the bridging glycoproteins between platelets and damaged endothelium. The molecular antigenicity of VWF:Ag can be analyzed by its multimeric distribution³ or by electrophoretic mobility.⁴ These analyses have helped the classification of von Willebrand's disease in various subtypes.¹⁻⁵ However, in a family, all members carry the same subtype but the severity of this condition is variable within the same kindred.

All these components show a parallel increase during stress, administration of estrogens, exercise, pregnancy, and certain medications such as desmopressin (Stimate). In some patients with von Willebrand's disease the factor VIII:C rises to normal levels, correcting the abnormally prolonged partial thromboplastin time.

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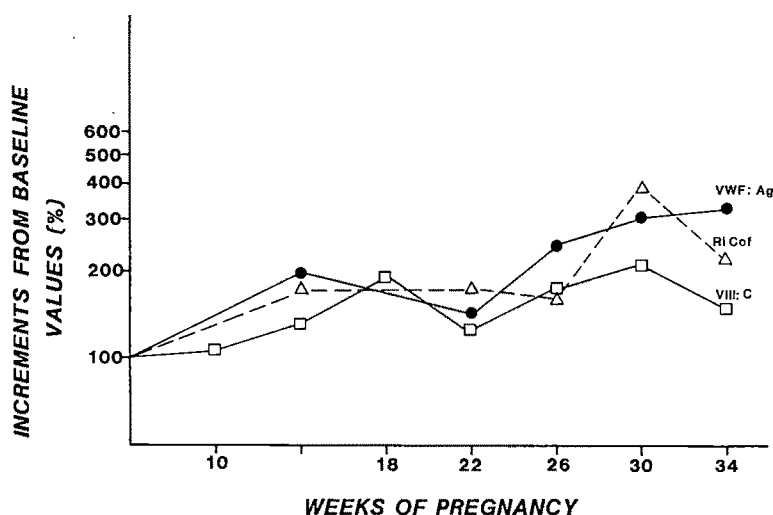


Fig. 1. Mean increments of VIII:C (□—□), VWF:Ag (●—●), and RiCof activity (Δ—Δ) in six patients (eight pregnancies) with von Willebrand's disease. The baseline value was considered to be 100%.

However, the other components might not show the same degree of response; therefore, one cannot evaluate the VIII:C level alone but must know the responses of VWF:Ag and RiCof as well as bleeding time. The only way to appropriately manage this problem is to electively test for the type and subtype of von Willebrand's disease that the patient suffers. One must know the outcome of previous pregnancies and the degree of severity and responses to events such as the administration of oral contraceptives. In some cases one must also examine the effect of infusion of desmopressin.

It is the purpose of this report to describe the changes of coagulation parameters during pregnancies in six subjects with von Willebrand's disease, the management during delivery, complications seen in either the mother or the offspring, and the diagnosis of this condition in newborn infants.

Material and methods

Patients. Six women, three white and three black with different subtypes of von Willebrand's disease, were followed up during eight pregnancies. The ages ranged from 18 to 38 years with an average of 26 years. The diagnosis of the bleeding disorder was made before pregnancy in three patients and in three others at the beginning of the second trimester. Three patients had type I disease and were very likely heterozygous for this condition (diagnosis based on laboratory data and family studies). Two patients (three pregnancies) had type IIA disease and one patient (two pregnancies) had type III disease (homozygous inheritance) judged by clinical symptoms, previous history, and laboratory studies (for definitions of laboratory types of von Wil-

lebrand's disease, please see Comment section). Four patients had severe von Willebrand's disease and two patients had a mild to moderate disorder. Since these patients are followed up in our bleeding disorder clinic, as soon as a patient was found to be pregnant she was invited to participate in this study; therefore, in three patients we had baseline laboratory information and subclassification of von Willebrand's disease before pregnancy. In three others, the patients were referred to us and the laboratory information had to be collected at the beginning of the second trimester of pregnancy.

Laboratory methods. The bleeding time was determined by means of a modified Ivy method with the Simplate (General Diagnostics) technique and a cuff pressure at 40 mm Hg.⁶ Venous bleed was collected by drawing specimens with a 19-gauge needle and a double syringe technique. The blood was then gently mixed with 3.8% sodium citrate in a ratio of 9:1 (blood to citrate). Platelet-rich plasma was obtained by centrifugation of blood at 500 rpm at room temperature for 20 minutes; the layer containing platelets was removed with a plastic Pasteur pipette. Platelet-poor plasma was obtained by further centrifugation of blood at 2500 rpm also at room temperature for 30 minutes. The activated partial thromboplastin time was measured with the use of platelet-poor plasma and a fibrometer (Becton Dickinson, Rutherford, NJ 07070).

Factor VIII coagulant (VIII:C) was performed by the one-stage method as described.⁷ von Willebrand factor antigen (VWF:Ag) was determined with a commercial antibody and the Laurell technique.⁸ The functional expression of von Willebrand factor was done as RiCof by the technique of Weiss et al.² with the use of increasingly diluted concentrations of patient plasma,

Table I. Pregnancies in von Willebrand disease, type of delivery, treatment, and maternal complications

Patient	Age (yr)	Type of von Willebrand's	Clinical severity	Pregnancy	Type of delivery	Treatment	Complications
P. N.	24	III	S	1	Vaginal	Whole blood, fresh plasma, cryoprecipitate	Severe vaginal bleeding; massive lumbar bleeding
				2	Elective cesarean section	Cryoprecipitate	Vaginal bleeding
J. L.	26	IIA	S	1	Vaginal	Packed red blood cells, cryoprecipitate	Significant postpartum bleeding
G. J.	28	IIA	S	2	Cesarean section	Cryoprecipitate; curettage	Placenta previa
				1	Elective cesarean section	Cryoprecipitate before and after delivery	None
C. C.	38	I	S	1	Cesarean section	Cryoprecipitate; desmopressin post partum	Membrane rupture; water intoxication after desmopressin
E. Y.	23	I	M	1	Cesarean section	Cryoprecipitate	None
C. V.	18	I	M	1	Vaginal	None	None

S = Severe; M = moderate.

normal washed platelets ($350/\text{mm}^3$ to $380,000/\text{mm}^3$), and ristocetin (Lenau, Copenhagen, Denmark) at final concentrations of 1.5 mg/ml.

Ristocetin-induced platelet agglutination was determined in platelet-rich plasma at platelet concentrations of $350/\text{mm}^3$ to $380,000/\text{mm}^3$, ristocetin at concentrations of 1.0, 1.25, and 1.5 mg/ml, and a Chronolog aggregometer attached to a recorder (Havertown, Pennsylvania). The electrophoretic mobility of VWF:Ag (crossed immunoelectrophoresis) was determined in either platelet-poor plasma or in its cryoprecipitated material as described.⁴ Normal pooled plasma was always used as a control.⁴

The measurements of factor VIII:C, VWF:Ag, and RiCof in the offspring were done in cord blood with the same anticoagulant and ratio (blood to anticoagulant). When the babies were older (>1 year) some of these tests were repeated. Control subjects and patients with von Willebrand's disease were receiving no medication at the time of testing and/or blood drawing.

Results

The clinical and laboratory changes seen in six patients with mild to severe forms of von Willebrand's disease before, during, and after eight pregnancies were analyzed. There were a total of eight deliveries and studies performed in cord blood and/or up to 1 year of age resulted in the diagnosis of five affected newborn babies. There were three mothers, one pregnancy each, with type I von Willebrand's disease, likely to be heterozygous for this condition. Two of them had a mild disorder and one was a more severely affected patient. There were two patients with type IIA. One of them was pregnant twice whereas the other one was studied during her second pregnancy since the first one resulted in a spontaneous abortion at 15 weeks. These two patients had a severe form of von Willebrand's

disease, characterized by spontaneous mucocutaneous bleeding, very low levels of functional von Willebrand factor, and markedly prolonged bleeding time. The electrophoretic mobility of VWF:Ag was abnormal; it was characterized by a faster migration. Of interest, the VWF:Ag for one patient (J. G.) showed a faster mobility than the other, indicating different degrees of a molecular abnormality. Finally, one patient, with what seems to be a rare example of type III von Willebrand's disease, was followed up during two complete pregnancies. A third pregnancy terminated in a spontaneous abortion. She seems to have a rare occurrence of homozygosity since her two parents and 14 other members of her family are mildly affected.

The age distribution of these six patients ranged from 18 to 38 years. Before being pregnant most of them were using oral contraceptives, which partially improved their clinical condition, as has been reported.⁹ Two of them have had one spontaneous abortion each, during the beginning of the second trimester.

As expected, the components of the factor VIII complex increased during pregnancy, reaching the highest values for RiCof and factor VIII:C at 25 to 32 weeks of pregnancy whereas VWF:Ag peaked at 35 weeks (Fig. 1). However, the rise of the factor VIII components was variable from patient to patient; those affected with type I showed marked improvement of factor VIII:C levels associated with significant elevations of factor VWF:Ag and RiCof. Two showed a normalization of the bleeding time but the most severely affected individual in this subgroup did not. The patients with type IIA and the subject with type III showed good elevations of factor VIII:C similar to those of the other patients but modest or no responses of factor VWF:Ag and RiCof. The bleeding time was unchanged from baseline values or it was minimally shortened.

Five patients underwent cesarean sections, three of

them because of obstetric considerations (membrane rupture, placenta previa, and profuse vaginal bleeding). The other two cesarean sections were elective and performed in an attempt to prevent bleeding complications in the fetus since the mothers were severely affected. One of them had a diagnosis of type IIA disease whereas the other one was the patient affected with type III von Willebrand's disease. Three deliveries were vaginal and none of them were induced. Despite prophylactic treatment, two of the vaginal deliveries were accompanied by profuse postpartum bleeding and required uterine reexploration and multiple transfusions of blood products and cryoprecipitate. One vaginal delivery, in a mildly affected individual, resulted in no complications. Table I summarizes the clinical data of these six patients.

Medical management. In preparation for the delivery, five patients during seven deliveries were treated with cryoprecipitate at a dose of 10 bags before and 10 bags every 8 to 12 hours in the first 24 to 48 hours of the postpartum period, with the dosage reduced to once daily for an additional period of 4 to 5 days. One patient (C. V.) was not given cryoprecipitate since the bleeding time was normal at the time of delivery.

In an attempt to improve the laboratory abnormalities and to reduce the need for cryoprecipitate, two patients were treated with a vasopressin analogue (desmopressin). The trade name of this medication is Stimate. One patient (C. C.) received three infusions of desmopressin at a dose of 0.3 $\mu\text{g/kg}$ every 18 hours during the postpartum period, whereas in another patient the desmopressin was given as a trial dose during the last month of pregnancy at a dosage of 0.4 $\mu\text{g/kg}$. In each case, the desmopressin was mixed with 50 ml of normal saline solution and infused in 30 minutes. The bleeding time, factor VIII:C, factor VWF:Ag, and RiCof were then determined at 30 minutes and 3 to 4 hours after the desmopressin infusion was stopped.

Complications. Several obstetric and medical complications were seen in the patients studied. Four pregnancies were complicated by massive bleeding. The cause of bleeding in one patient was a placenta previa. Another patient, with type III von Willebrand's disease, developed an atonic uterus requiring hormonal treatment. The third severe hemorrhage developed in a patient with type IIA disease who was receiving cryoprecipitate. It is possible that the cryoprecipitate given to her had reduced amounts of the large polymers of factor VWF:Ag. However, the usual requirements of cryoprecipitate to correct the bleeding time are larger than those of other patients with a similar condition. Finally, the last patient, who was delivered vaginally, developed uterine bleeding associated with significant lumbar hematomas. During the time of the study there were two spontaneous abortions in this group of six

patients but neither of them was associated with severe bleeding. Therefore, the total number of pregnancies was 10; however, since the spontaneous abortion was at ≤ 15 weeks, these two were not taken into consideration in this study.

The two patients who received desmopressin had complications related to water retention; in one of them, after three doses (given every 18 hours), the sodium level dropped to 108 mEq/L, which precipitated the appearance of a grand mal seizure. The other patients received a single test dose but developed premature labor believed to be secondary to water retention. These medical and obstetric complications will be the subject of a detailed report.

Results of offspring. Of the eight newborn babies (five boys, three girls) we have laboratory data on seven. Two babies did not inherit the disorder since the laboratory studies did not show low levels of RiCof and factor VWF:Ag or prolonged bleeding time (performed on blood from the heel of the newborn baby). Five patients were affected and, when compared with laboratory data of the mothers, three of them showed higher values of RiCof. The other two babies, offspring of women with type IIA disease, have values of RiCof equal to those of their mothers (Table II). Only one newborn baby, the second offspring of the patient with type III von Willebrand's disease (P. N.), developed a subcutaneous head hematoma. No treatment was given to him and we believe that this bleeding complication was the result of strong uterine contractions before cesarean section; the laboratory data of the baby's blood suggests that he was less affected than the mother. Only time will tell us if these new patients will present with the same clinical severity or amount of factor VWF:Ag and RiCof will differ with increasing age.

Comment

The recently developed laboratory tests applied in the diagnosis of von Willebrand's disease have helped the understanding of the molecular abnormalities found in this condition. Their application has also helped in the clarification of genetic patterns and in the classification of this disorder into subtypes (or variants). From a practical point of view, these disorders could be classified as quantitative and/or qualitative conditions.

Type I is usually a mild condition characterized by a parallel diminution of VIII:C, VWF:Ag, and RiCof and a mild prolongation of the bleeding time and is usually seen in the heterozygote; this is the type of von Willebrand's disease that usually responds to the infusion of desmopressin¹⁰ and administration of estrogens⁹ and shows a significant laboratory improvement during pregnancy.¹¹ Type III is a rare form of a quantitative abnormality of factor VIII usually seen in

Table II. Baseline values in mothers affected with von Willebrand disease and their offspring

Patients	Bleeding time (min)	Partial thromboplastin time (sec)	Mother (% of normal)				CIE of von Willebrand factor
			VIII:C	VWF:Ag	RiCof	RIPA (% slope) (1.5 mg/ml)	
Type III P. N.	>15	49	20	<5	<5	0	—
Type IIA J. L.	>15	47	49	12	12	21	Faster
G. J.	>15	42	43	46	8	8	Faster
Type I C. C.*	>15	46	29	6	3	20	Normal
E. Y.*	8	31	218	51	107	47	Normal
C. V.*	13.5	40	92	39	61	36	Normal
Normal values	3.7	35-40	50-150	50-150	60-150	60-70	—

CIE = Crossed immunoelectrophoresis. RIPA = Ristocetin-induced platelet aggregation.

*Patient was pregnant.

the homozygote. The clinical manifestations of bleeding are severe since no measurable RiCof or VWF:Ag can be assessed. These patients may be offspring of consanguineous marriages or of two unrelated heterozygous individuals. Few such patients are known to exist and only extensive family studies will help in establishing the inheritance pattern. We feel that one of our patients (P. N.) is such an example. Limitation of space does not allow us to show the family pedigree, which includes 14 affected subjects. In addition to the very low levels of RiCof, the levels of factor VWF:Ag and factor VIII:C are low to absent. The bleeding time is markedly prolonged and the patient usually responds to the administration of cryoprecipitate. However, no experience has been reported after the administration of desmopressin. In our patient the administration of desmopressin was associated with a modest elevation of VWF:Ag, RiCof, and VIII:C levels and no significant correction of the bleeding time.

In the qualitative subgroups, because of improper release, assembly, or structure of VWF:Ag polymers, type IIA is the most common. It is characterized by a severe bleeding disorder, markedly prolonged bleeding time, and very low levels of RiCof activity. The factor VIII:C and VWF:Ag are variable. If tested by immunoelectrophoresis, VWF:Ag shows an increased electrophoretic mobility because of the absence of high molecular weight polymers. It is very likely that different degrees of severity will be found in this subgroup of patients as we have observed. Treatment includes the administration of cryoprecipitate, which contains the needed high molecular weight polymers. The infusion of desmopressin will result in a minimal correction of laboratory abnormalities. Since the molecular disorder is characterized by variable amounts of the polymers some patients will clinically improve, whereas

others will not have correction of the bleeding time.¹² Few previous reports of the response during pregnancy in this subtype have been published. Type IIB, in which the patient produces polymers with increased affinity for platelets, is even less common than IIA. One case of a pregnant patient with type IIB was reported by Hanna et al.¹³ in 1981. The small experience with desmopressin in type IIB suggests it should be avoided since it may cause thrombocytopenia.

Our study clearly shows that the components of factor VIII complex have a synchronous gradual increase during pregnancy with peak values at the middle of the third trimester as shown by Kasper et al.,¹⁴ when the response of factor VIII:C was studied during pregnancy. However, more significant than the correction of the partial thromboplastin time and/or factor VIII:C is the response of RiCof activity, which has a closer relationship with the correction of the bleeding time or clinical bleeding. Thus the monitoring of patients with von Willebrand's disease during pregnancy should include several determinations of the bleeding time and RiCof as well.

Most of the experience in the management of pregnant individuals with von Willebrand's disease has been obtained by studying patients with a mild to moderate disorder.¹¹⁻¹³ In these cases as well as our patients with type I disease, there was a progressive improvement of the reduced components as well as a correction of the bleeding time during the pregnancy.^{11-13, 15} Lesser experience, however, has been reported in the management of severe cases of von Willebrand's disease during pregnancy. One of the main reasons for lack of clinical and laboratory improvement could be due to the fact that in type II von Willebrand's disease the rate of production of the abnormal protein remains intact or increases.¹⁶ In spite of increased production, there re-

Offspring (% of normal)			Complications
VIII:C	VWF:Ag	RiCof	
Normal	8 11	31 9	None Scalp hematoma
—	—	—	None
40	8 140	13 17	None None
17	14	2	None
61	—	—	None
—	—	—	None
60-150	60-150	60-150	

mains an absence of the normal type of VWF:Ag molecules. In one of the two cases reported by Lipton et al.¹⁶ and in the case reported by Adashi,¹⁷ no improvement of factor VWF:Ag and RiCof was seen, whereas factor VIII:C increased to approximately 200% of baseline values. This observation was also present in our patients with type IIA and type III disease.

Of the six patients in our study, four patients were considered to have severe forms of this disorder. Three of them showed no correction of the bleeding time during pregnancy whereas the fourth patient, known to have type I disorder, improved. Patients in whom the bleeding time was not corrected were those individuals with either type IIA or type III. This lack of correction therefore is in agreement with that in previously reported cases.^{16,17} Of interest is the observation that in most patients the partial thromboplastin time and factor VIII:C improve or are completely normal. This applies to our cases as well to those published elsewhere.^{11,17}

It is important to stress the fact that the protein measured as factor VWF:Ag and RiCof does not cross the placenta and the newborn values are independent of levels obtained in the mother. Therefore in type IIA the fetus could be more or less affected than the mother. Thus, if we are dealing with subjects with severe type IIA and III disease, it is recommended to perform an elective cesarean section in order to avoid bleeding complications in the newborn infant. One of our newborn babies had a subcutaneous hematoma, which we feel was due to early uterine contractions, since the delivery was a cesarean section after the beginning of labor. In addition, the pregnant patient who undergoes a cesarean section is treated under controlled conditions with the help of cryoprecipitate. Vaginal deliveries should be reserved for patients with milder conditions (type I) since they improve during pregnancy and can be monitored by the bleeding time.

The use of cryoprecipitate and/or desmopressin in this latter group of patients depends on the degree of severity and on the bleeding time. It is also important to stress that the bleeding complications during vaginal delivery or cesarean section in patients with von Willebrand's disease are not related to the amount of factor VIII:C, which can be high normal even when the bleeding time is markedly prolonged. These patients should be monitored by the bleeding time.

Another important point to consider at the time of delivery is to obtain an anticoagulated sample of cord blood (citrate blood—blue-top tube) to measure various coagulant and immunologic parameters that could be abnormal in patients with von Willebrand's disease. The red blood cells should be spun off and the plasma sample kept on ice until assay. However, if the blood has clotted, the diagnosis of von Willebrand's disease in the newborn infant could still be done by measuring VWF:Ag and/or RiCof. Five of eight newborn babies were affected in our group of patients. At present we are evaluating whether the in vitro endothelial cell growth patterns of the umbilical cords from these infants correlate with the amount and molecular composition of VWF:Ag present in the cord blood as previously reported in one patient.¹⁸ These data, however, are still preliminary.

Since the laboratory evaluation of these patients is laborious and often must be repeated over time, and since the differentiation of the subtypes of von Willebrand's disease may make a great difference in suggested treatment, it is imperative that young women with a family or personal history of a bleeding disorder be evaluated as early as possible, preferably before pregnancy, so that some genetic counseling may be done. For the woman discovered to have a hemostatic disorder during pregnancy diagnostic testing must be begun as soon as possible. For the severely affected patient, very close collaboration between obstetrician and hematologist will be necessary to avoid hemorrhage and bring the mother and child safely through delivery.

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Effects of meclofenamate and acetaminophen on abdominal pain following tubal occlusion

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To test the hypothesis that the postoperative abdominal pain of tubal occlusion is mediated by prostaglandins, the effects of meclofenamate, a potent inhibitor of cyclooxygenase, on postoperative analgesia and incidence of abdominal pain were compared with those of acetaminophen, a weak inhibitor of prostaglandin activity. One hundred patients undergoing tubal occlusion under local anesthesia were studied. The patients were randomly divided into four equal groups: (1) control; (2) acetaminophen, 1300 mg; (3) meclofenamate, 100 mg; (4) meclofenamate, 200 mg. The fallopian tubes were occluded by electrocautery in 47 patients and by application of Falope rings in 53 patients. Both acetaminophen and meclofenamate provided substantial analgesia for 4 hours after the operation ($p < 0.05$). Meclofenamate reduced the incidence of abdominal pain by one half ($p < 0.02$), but acetaminophen did not. These results suggest that a portion of pain relief achieved by meclofenamate may be due to suppression of myosalpingian and/or myometrial contractions, a process mediated by prostaglandins. (*AM J OBSTET GYNECOL* 1986;155:624-9.)

Key words: Acetaminophen, meclofenamate, Falope ring, electrocautery, abdominal pain

The postoperative abdominal pain and discomfort that follow laparoscopic tubal occlusion may be severe enough to delay the return of a patient to regular ac-

tivity for a day or two. Recently, topical application of various local anesthetics in different concentrations and amounts has been successfully employed for this procedure. Although these anesthetic techniques appear to offer some protection in the postoperative period, for example, for up to 2 hours,^{1,2} the overall incidence of abdominal pain and discomfort remains high.

The lower midabdominal pain that follows tubal occlusion is usually colicky and may radiate to the lower back and upper thighs. Accompanying symptoms include nausea and occasionally vomiting. These symp-

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Table I. Intraoperative pain score

Group	Procedure	Pain score (number of patients in each category)				
		1	2	3	4	5
1	Control					
	Falope ring	2	3	7	2	0
	Electrocautery	1	2	4	2	2
2	Acetaminophen, 1300 mg					
	Falope ring	3	5	4	1	0
	Electrocautery	1	2	4	4	1
3	Meclofenamate, 100 mg					
	Falope ring	4	7	1	0	0
	Electrocautery	1	6	4	2	0
4	Meclofenamate, 200 mg					
	Falope ring	4	8	3	1	1
	Electrocautery	2	2	4	0	0

toms are similar to those experienced by patients with dysmenorrhea, suggesting that a mechanism involved in postoperative abdominal pain following tubal occlusion may be a prostaglandin-mediated change in myosalpingian and/or myometrial activity.

Our clinical trial was designed to evaluate whether meclofenamate, an analgesic and a potent prostaglandin synthetase inhibitor,^{3,4} relieves postoperative pain following tubal occlusion, and if it does, whether some of the pain relief thus obtained is due to the effect of the drug on prostaglandins. For the purpose of evaluating the latter objective, the effect of meclofenamate on postoperative lower midabdominal pain was compared with that of acetaminophen, an analgesic with a weak antiprostaglandin activity.⁵

Material and methods

One hundred patients who were classified by means of the American Society of Anesthesiologists Classification as ASA I and II and who underwent laparoscopic bilateral tubal occlusion under local anesthesia for voluntary sterilization participated in this study. An informed consent, approved by the University Human Studies Committee, was obtained for all patients. Patients were randomly divided by drawing a number into four groups with 25 patients in each group. Group 1 patients received 10 mg of diazepam, group 2 received 10 mg of diazepam and 1300 mg of acetaminophen, group 3 received 10 mg of diazepam and 100 mg of meclofenamate, and group 4 received 10 mg of diazepam and 200 mg of meclofenamate. The medication was taken with a sip of water approximately 1 hour before the operation. Since no study comparing the analgesic potency of acetaminophen with meclofenamate was found in the literature, the dose of acetaminophen was selected empirically based on the observation that aspirin was as effective as meclofenamate in the treatment of symptoms of rheumatoid arthritis in the above dose ranges.⁶ Neither patients

nor observers were told the identity of the premedication.

The routine operative procedure practiced by our surgical colleagues included insertion of a Cohen's intrauterine cannula for uterine manipulation. To provide analgesia during the manipulation, 20 ml of 0.5% lidocaine was injected through the cannula. For periumbilical infiltration, 10 to 20 ml of 0.5% lidocaine was used. Following inflation of the abdominal cavity with nitrous oxide, each fallopian tube was sprayed with 10 ml of 0.5% bupivacaine under direct laparoscopic vision. The fallopian tubes were occluded randomly as a part of an ongoing study either by application of Falope rings (those patients having an odd number as the last digit of their hospital record number) or electrocautery (those with an even number).

The intraoperative pain was evaluated by an anesthetist and graded on a scale of intensity ranging from 1 to 5, which was interpreted as follows: 1 = no pain; 2 = mild pain; 3 = moderate pain, often requiring an intravenous injection of fentanyl; 4 = severe pain, often requiring both fentanyl and 50% nitrous oxide for a few minutes; 5 = very severe pain, requiring general anesthesia. The postoperative pain was graded on a similar scale: 1 = no pain; 2 = mild; 3 = moderate, requiring medication; 4 = severe; and 5 = very severe.

Patients were observed in the recovery room for 2 hours before being discharged home. Pain severe enough to require medication (pain score ≥ 3) was treated empirically with 2 to 4 mg of morphine sulfate intravenously, 650 mg of acetaminophen, or 100 mg of meclofenamate. At the time of discharge, patients were given a prescription for 100 mg of meclofenamate. Patients were followed up by telephone on the evening of operation, on the first postoperative day, and on the second postoperative day with regard to the time of first intake of pain medication and the nature of pain for which the medication was taken. When medication

Table II. Recovery room pain score

Group	Procedure	Pain score (number of patients in each category)				
		1	2	3	4	5
1	Control					
	Falope ring	2	1	5	6	0
	Electrocautery	5	1	1	4	0
2	Acetaminophen, 1300 mg					
	Falope ring	6	4	1	2	0
	Electrocautery	3	3	2	4	0
3	Meclofenamate, 100 mg					
	Falope ring	4	4	3	1	0
	Electrocautery	6	4	2	1	0
4	Meclofenamate, 200 mg					
	Falope ring	9	5	2	1	0
	Electrocautery	3	4	1	0	0

Table III. The cumulative number of patients who required pain medication primarily for abdominal cramp

Group	Procedure	n	Postoperative hours							
			0.5	1	2	4	8	12	24	48
1	Control									
	Falope ring	12	7	7	8	9	9	10	11	11
	Electrocautery	13	6	6	6	9	9	10	11	11
	Total	25	13	13	14	18	18	20	22	22
2	Acetaminophen, 1300 mg									
	Falope ring	13	2	2	3	6	8	9	9	9
	Electrocautery	12	3	3	4	4	6	6	6	6
	Total	25	5	5	7	10	14	15	15	15
3	Meclofenamate, 100 mg									
	Falope ring	12	2	2	2	3	4	6	6	6
	Electrocautery	13	0	1	1	1	1	3	3	3
	Total	25	2	3	3	4	5	9	9	9
4	Meclofenamate, 200 mg									
	Falope ring	16	1	1	1	5	7	7	8	8
	Electrocautery	9	0	1	1	2	3	3	3	3
	Total	25	1	2	2	7	10	10	11	11

was taken for more than one type of pain, the medication was considered to have been taken for the worst type of pain.

To compare the incidence of abdominal cramp and/or low back pain during the recovery room period (the first 2 postoperative hours), those patients who developed both abdominal cramp and other types of pain were considered to have abdominal cramp. Those who received pain medications for pain other than abdominal cramp, that is, incisional pain, pain in the chest, neck, and shoulder, and sharp lower abdominal pain located in either lower quadrant that was described as being different from the usual menstrual cramp, were excluded from analysis, since such medications could influence the incidence and/or patient's perception of abdominal cramp.

All multidimensional tables were analyzed with use of Biomedical Data Program-4F. Goodness of fit and marginal and partial associations were also included in

the analysis of three-dimensional tables. The *p* values quoted were the largest values when all tests were used. For comparison of such variables as age, weight, height, operation time between groups or subgroups, analysis of variance was performed with STAN (Statistical Consultants, Inc., Lexington, Kentucky).

Results

The mean age, weight, and height were as follows: 26.7 ± 0.9 (SEM) years, 69.1 ± 3.8 (SEM) kg, and 165.1 ± 1.0 (SEM) cm in group 1; 26.2 ± 0.8 years, 64.5 ± 2.9 kg, and 160.0 ± 1.0 cm in group 2; 27.2 ± 1.1 years, 67.7 ± 3.6 kg, and 162.6 ± 1.5 cm in group 3; and 27.0 ± 1.0 years, 68.2 ± 3.6 kg, and 162.6 ± 1.0 cm in group 4. The distributions were similar among the groups. Obstetric history, menstrual cycle and its duration, occurrence of dysmenorrhea, and the type of contraceptive used were not significantly different among the groups. Sixteen patients were in

Table IV. The incidence of abdominal cramp and/or low back pain in the first 2 postoperative hours (recovery room)

Group	Procedure	n	Abdominal cramp*	
			n	%
1	Control	23	16	69.6
2	Acetaminophen, 1300 mg	24	14	58.3
3	Meclofenamate, 100 mg	22	6	27.3
4	Meclofenamate, 200 mg	25	7	28.0

*Pain score ≥ 2 .

their menstrual period at the time of operation: two were in group 1, five were in group 2, two were in group 3, and seven were in group 4. The mean operative time was 28 ± 5 (SD) minutes with the range of 20 to 45 minutes. There was no association between these variables and intraoperative or postoperative pain. Fallopian tubes were occluded by electrocautery in 47 patients and application of Falope rings in 53 patients. The occlusion techniques were evenly distributed in groups 1, 2, and 3. In group 4, 16 patients received Falope rings and nine patients had electrocautery.

General anesthesia (pain score = 5) was required in four patients: two in group 1 and one each in groups 2 and 4. The highest intraoperative pain score occurred at the time of tubal occlusion, usually lasting for a few seconds. The score was not significantly different among the groups, but in each of the four groups the pain score was significantly higher ($p < 0.05$) in patients whose fallopian tubes were occluded by electrocautery rather than with Falope rings (Table I). In the first 2 postoperative hours (the recovery room period) the technique of occlusion had no effect on the pain score in any of the four groups (Table II).

Premedication with acetaminophen and meclofenamate reduced the number of patients who required pain medication (pain score ≥ 3) in the recovery room significantly ($p < 0.01$). Of the patients requiring pain medication in the first 2 postoperative hours (recovery room), 16 were in the control group, nine were in group 2, seven were in group 3, and four were in group 4. Meclofenamate was more effective than acetaminophen in reducing the incidence of pain ($p < 0.05$). The analgesic effect of acetaminophen and meclofenamate was evident during the first 4 postoperative hours ($p < 0.01$).

The premedication also reduced the incidence of abdominal cramp (pain score ≥ 3) significantly ($p < 0.01$). Premedication with acetaminophen and two different doses of meclofenamate (100 and 200 mg) reduced the incidence to one half and approximately one fifth, respectively, in the first 2 postoperative hours

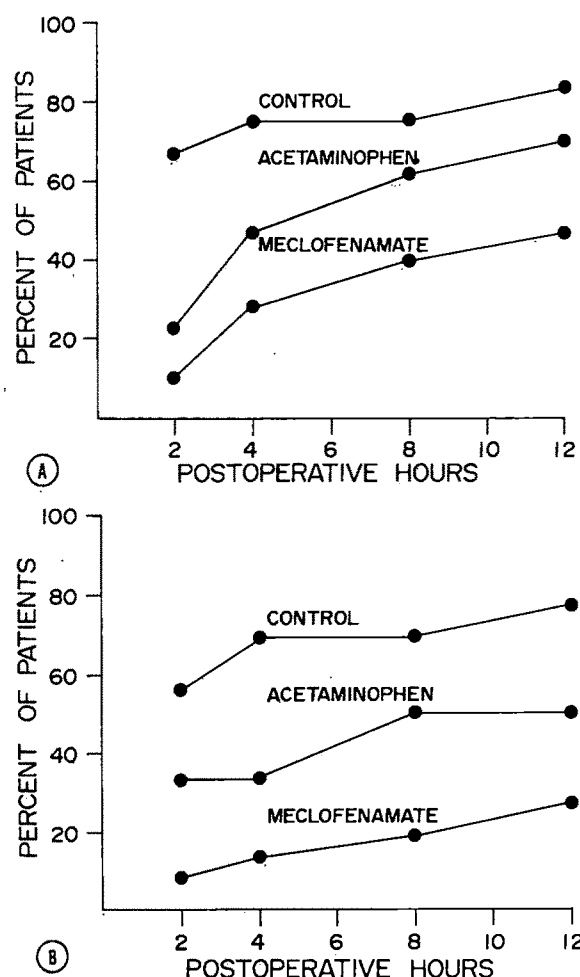


Fig. 1. A, The cumulative incidence of abdominal pain in the Falope ring group. B, The cumulative incidence of abdominal pain in the electrocautery group.

(Table III). The difference in the incidence between the acetaminophen and meclofenamate groups was significant ($p < 0.05$). There was no significant difference in the incidence between the meclofenamate groups. The incidence of abdominal cramp of all intensities (pain score ≥ 2) in the first 2 postoperative hours (the recovery room period) was 69.5%, 58.3%, 27.3%, and 28.0% in groups 1 to 4, respectively (Table IV). The incidence was significantly lower in the meclofenamate groups ($p < 0.025$). There was no significant difference in the incidence between the control and the acetaminophen group. There was no significant difference between the meclofenamate groups.

During the 48-hour postoperative follow-up period, 85 patients (85.0%) required medication for pain. Fifty-seven of these 85 patients (67.1%) required medication primarily for abdominal pain and for low back pain (abdominal cramp). Eight of the 85 patients (9.4%) had taken pain medication by the end of the 12th postoperative hour. In the control group, 24 of 25 patients

required pain medication during the follow-up period (Table III). The pain occurred early in this group. Despite local anesthetization of fallopian tubes, 14 (58.3%) and 16 (66.7%) of the 24 patients took pain medication within 30 minutes and 2 hours of operation, respectively. Twenty-two of the 24 patients (91.7%) took medication for abdominal cramp or primarily for abdominal cramp during the follow-up period. The patients usually described the pain as similar to labor pain or menstrual pain.

The technique of tubal occlusion had no effect on the cumulative incidence of abdominal cramp (pain score ≥ 3) in the control patients throughout the 48-hour follow-up period (Table III). The cumulative numbers of patients who took medication were identical in the ring and electrocautery groups after the first 2 postoperative hours. In the acetaminophen and meclofenamate groups the technique of occlusion did not affect the incidence of abdominal cramp in the first 2 postoperative hours ($p < 0.2$). However, the incidence increased steadily thereafter in the ring group, approaching that in the control group by the end of the twelfth postoperative hour (Fig. 1, A and B). The increase in the incidence of abdominal cramp was less in the electrocautery group. At all stages, the incidence in the acetaminophen and meclofenamate groups was significantly less than that in the control group ($p < 0.05$) and the incidence in the meclofenamate groups was significantly less than that in the acetaminophen group ($p < 0.05$). Within the acetaminophen group there was no statistically significant difference in the incidence of abdominal cramp between the techniques of occlusion. Within the meclofenamate group there was a marginal significance in the difference resulting from the technique of occlusion ($p < 0.1$).

Comment

Patients tolerated laparoscopic tubal occlusion well under local anesthesia. However, despite topical anesthesia of the fallopian tubes, the incidence of pain in the control group of patients was disturbingly high in the immediate postoperative period and the pain intensity was substantial for a relatively minor procedure. Approximately two thirds of the patients required pain medication within 2 hours of operation, and by the end of the twelfth postoperative hour, 96% of the patients had required medication for pain. The majority of pain was abdominal cramp (91.6%) located in the lower mid-abdomen and/or low back pain, and the patients typically described the pain as being similar to that experienced during labor or menstruation.

Prostaglandins E_2 and $F_{2\alpha}$ in the endometrium and myometrium are intimately involved in the initiation and maintenance of myometrial contractions during labor, menstruation, and dysmenorrhea. The prosta-

glandins are also present in the human endosalpinx and released in response to various stimuli, for example, trauma, irritation, and distension.⁷⁻⁹ Thus an appealing hypothesis would be that prostaglandins released in the endosalpinx in response to trauma and ischemia of the fallopian tubes act on the myosalpinx and possibly the myometrium, producing painful contractions. Recently a report demonstrated that preoperative administration of indomethacin, a potent prostaglandin synthetase inhibitor, decreases the postoperative pain of tubal occlusion significantly.¹⁰ Nonopioid antiinflammatory analgesics, however, are surprisingly effective for the control of postoperative pain.¹¹ The salicylates may be as effective as narcotics for the relief of pain after less painful and superficial operations. In a study,¹² indomethacin reduced the postoperative pain scores by more than two thirds and narcotic requirements by more than one half following major abdominal operations.

Acetaminophen and meclofenamate provided substantial pain relief comparable to that reported previously, up to 4 hours after tubal occlusion. Both drugs reduced the postoperative requirement of pain medication in general and that for abdominal cramp significantly. Meclofenamate was significantly more effective than acetaminophen in reducing these requirements. It is likely that the doses of acetaminophen and meclofenamate used empirically in this trial may not have been equipotent, since such doses have not been established between the drugs. Thus the apparently more effective reduction of postoperative pain with meclofenamate could have been a dose-dependent phenomenon. However, in the first 2 postoperative hours, that is, the recovery room period, meclofenamate reduced the incidence of abdominal cramp of all intensities to less than one half of that in the control group, but acetaminophen did not reduce the incidence. This observation indicates that the action of meclofenamate is more specific to abdominal cramp and suggests that meclofenamate may achieve postoperative pain relief, in part, by its specific action on abdominal cramp following tubal occlusion.

The incidence of postoperative abdominal pain has been reported to be higher when the fallopian tubes were occluded with Falope rings than by electrocautery.^{13, 14} In this trial the technique of tubal occlusion did not influence the incidence of abdominal cramp severe enough to require pain medication in the first few postoperative hours in any of the four groups. In the control group the majority (81.8%) of patients had taken or were taking pain medication for abdominal cramp by the fourth postoperative hour. There was no difference during the subsequent observation in the cumulative incidence of abdominal cramp resulting from the techniques used. The drug pretreatment sub-

stantially reduced the incidence of abdominal cramp in the first 2 postoperative hours. However, in those patients who received Falope rings for occlusion, abdominal cramp continued to develop thereafter and the incidence approached that in the control group by the twelfth postoperative hour. The increase in the incidence of abdominal cramp was much less in patients whose fallopian tubes were occluded by electrocautery, and there were significant differences in the incidence among the groups during the entire follow-up period. The overall incidence was highest in the control group and lowest in the meclofenamate group. The prolonged effectiveness beyond the half-life of these drugs suggests that the differences in the incidence of abdominal cramp may be due to the technique of tubal occlusion and that the effect of insult to the fallopian tubes by electrocauterization is short-lived, but with the application of Falope rings, it lasts longer, that is, several hours or more. However, within the acetaminophen or meclofenamate groups the difference in incidence of abdominal cramp resulting from surgical techniques of occlusion was not statistically significant, which was probably due to the large group variation in a three-way analysis.

Specific effectiveness of meclofenamate on abdominal pain that follows tubal occlusion indicates that there is a difference in the mechanisms of action between acetaminophen and meclofenamate. The observation that topical anesthetization of the fallopian tubes was adequate for the occlusion procedure but inadequate for the postoperative pain suggests that the ascending sensory nerve impulses originating at the sites of occlusion may be a rather minor factor in the etiology of postoperative pain experienced by patients following bilateral tubal occlusion. A recent controlled randomized study demonstrated that topical anesthesia of fallopian tubes before occlusion did not reduce postoperative pain significantly.¹⁵ The quality of pain was similar to that of menstrual or labor pain. These observations do not prove but are consistent with the assumption that the underlying mechanism of abdominal pain may be prostaglandin-mediated myosalpingian and myometrial contractions. It has been proposed that the high incidence of abdominal pain in the Falope ring technique is due to slow necrosis of the tissue and nerves at the site of occlusion. An alternative explanation might be that ischemia and/or mechanical compression in the viable endosalpinx continues to synthesize and release prostaglandins, whereas the synthesis ceases in the tissue following electrocautery.

Meclofenamate is readily absorbed after oral intake, resulting in peak plasma levels in 30 minutes to 1 hour.¹⁶

It is a short-acting drug with a half-life of 2 hours. A longer-acting drug, for example, indomethacin or naproxen, would provide a longer period of protection against postoperative abdominal pain following tubal occlusion.

We wish to thank Susan L. Riley, R.N., C.R.N.A., for the care of our patients and Kathleen A. Robbins, R.N., for her administrative assistance.

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Telephone transmission of fetal heart rate monitor data

The experience at the University of Connecticut Health Center

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This report reviews the experience at the University of Connecticut Health Center using the Fetal Assessment Consultative Transmission Service (FACTS) system during a 2½-year period. This system, which permits direct transmission of antepartum and/or intrapartum fetal heart rate tracings via a telephone line, allows the obstetric staffs of smaller community hospitals to obtain an immediate consultation from the University of Connecticut Health Center on a 24 hour per day basis. A total of 511 fetal heart rate tracings were analyzed. Two hundred forty-five were intrapartum, 206 antepartum, and 60 were transmitted for educational purposes. The results indicate an imperative need for such a service from a tertiary care center to improve the quality of regional perinatal care and to determine the future direction of the regional educational program for physicians and nurses. (AM J OBSTET GYNECOL 1986;155:630-4.)

Key words: Telephone transmission, fetal heart rate, FACTS

During the past decade there have been dramatic changes in the methods used to assess fetal condition. These changes are obviously related to the widespread use of electronic fetal heart rate monitoring, which has become an integral part of the intrapartum as well as antepartum fetal surveillance. The presence of a reassuring fetal heart rate pattern during the intrapartum or antepartum period is an indication for expectant management. The presence of an abnormal intrapartum fetal heart rate pattern may be an indication for maternal medical treatment (for instance, positional changes, oxygen administration, discontinuation of oxytocin), further investigation (for instance, fetal acid-base assessment), or intervention (for instance, prompt delivery by cesarean section or forceps). However, fetal heart rate patterns are occasionally confusing and not easy to interpret. Under these circumstances a prompt transmission of the fetal heart rate tracing from a regional hospital to a tertiary care center for an immediate and expert consultation would seem appropriate.

Earlier reports have described the transmission of fetal heart rate tracings via a Xerox 400 Telecopier^{1,2} or telemetry.³ The Xerox method, however, had major disadvantages, such as limited transmission time (only

an 8-minute portion of the fetal heart rate tracing could be transmitted), lack of clarity, and inability to store data for retrospective analysis.

The Fetal Assessment Consultative Transmission Service (FACTS) system was introduced by the University of Connecticut Health Center, Division of Maternal-Fetal Medicine, and has been in full operation since January 1, 1983. This program permits assessment of continuous recordings of fetal heart rate and uterine activity data, which are transmitted to the University of Connecticut via telephone line from small community hospitals (in the region) for interpretation and consultation. The system provides a clear and reliable hard-copy record (Fig. 1) of the transmitted data, which allows us to offer an immediate emergency consultation for any hospital irrespective of location or distance. Most of the current models of monitors can be used for transmission.

The purpose of the present communication is to report our 2½ years' experience with the FACTS system and to discuss the impact of this service in improving perinatal outcome in the region.

Material and methods

From January 1, 1983, to June 30, 1985, 17 hospitals throughout North Central Connecticut and Western Massachusetts used the FACTS system. Each local hospital is equipped with one or more transmitting units (Litton Model DT100). One receiving unit (Litton Model DR100) is placed in the Maternal-Fetal Intensive Care Unit, University of Connecticut Health Center, and two additional receiving units are available to fac-

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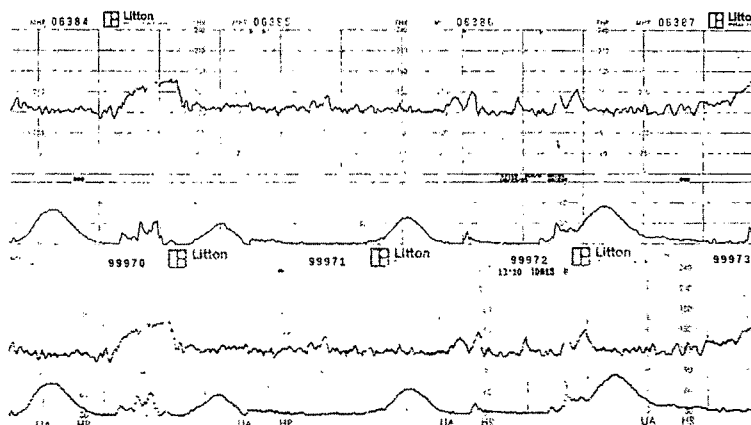


Fig. 1. The *upper panel* represents an external fetal heart rate tracing from a patient in early labor, and the *lower panel* represents the transmitted hard copy. Note the similarity and clarity of the obtained information.

ulty members on call. The service operates on a 24 hour per day basis.

The transmission process is simple. The transmitting unit is connected to a telephone line and a fetal heart rate monitor. Once electronic fetal heart monitoring begins, the transmitting unit is activated and stores fetal heart rate and uterine contraction data. Maximum storage capacity is 2 hours. When a telephone call requesting a consultation is received at the University of Connecticut Health Center, the patient's history is obtained and logged into our records. Then, using the telephone connected to the transmitting unit, the person who requests the transmission dials a number assigned to the receiving unit, activates the transmitting unit, and the transmission begins. The stored data are received at 12 times real time; therefore, a 2-hour retrospective record is transmitted in 10 minutes. After the transmission of the stored data, the unit continuously updates the information (on-line data) for as long as the interpreter feels it is necessary.

After the data are interpreted, the obstetric health care provider in the transmitting hospital is offered advice based on the information obtained from the fetal heart rate tracing and the clinical circumstances of each case. The perinatal nursing specialists at the health center are responsible for the fetal heart rate tracings that are transmitted for educational purposes. The maternal-fetal medicine faculty members are responsible for fetal heart rate tracings transmitted for consultations. No written follow-up of the consultation was provided to the transmitting hospital.

The transmitted antepartum fetal heart rate tracings consisted of nonstress tests or contraction stress tests. The nonstress tests were interpreted as (1) reactive, (2) nonreactive, or (3) unsatisfactory. The criteria used for the definition of a reactive nonstress test were two or

more accelerations (of at least 15 bpm above the baseline lasting at least 15 seconds) within a 10-minute period. When these criteria were not fulfilled the test was interpreted as nonreactive. The contraction stress tests were interpreted as (1) negative, (2) positive, or (3) suspicious according to the criteria of Freeman and Garite.⁴ Our recommendations for the transmitted antepartum fetal heart rate tracings were: delivery, repeat the testing within a week, further evaluation with the fetal biophysical profile, or maternal transport to our center (in the presence of prematurity or other complications requiring expert care in a tertiary center).

The transmitted intrapartum fetal heart rate tracings had been obtained by using external and/or internal fetal heart rate monitoring. The intrapartum fetal heart rate tracings were interpreted as (1) normal, (2) suspicious, (3) threatening, or (4) ominous. Fetal heart rate patterns were interpreted as suspicious in the presence of one of the following: (1) mild to moderate variable decelerations, (2) fetal tachycardia (>160 bpm) with good variability, (3) fetal bradycardia (<120 bpm) with good variability, and (4) nonrepetitive late decelerations with good variability. Threatening fetal heart rate tracings were diagnosed in the presence of (1) severe variable decelerations, (2) fetal tachycardia with decreased variability (3 to 5 bpm), (3) repetitive late decelerations, and (4) decreased beat-to-beat variability not related to analgesia. Fetal heart rate patterns were interpreted as ominous in the presence of one of the following: (1) absent beat-to-beat variability (<2 bpm) not related to analgesia, (2) severe variable decelerations with absent variability, (3) fetal tachycardia (>160 bpm) with absent variability, (4) persistent late decelerations with absent variability, and (5) fetal bradycardia (<120 bpm) with decreased or absent variability.

Based on this interpretation and the clinical circum-

Table I. Recommended management for antepartum fetal heart rate transmissions (total number = 206)

<i>Management</i>	<i>No.</i>	<i>%</i>
Delivery	24	11.6
Repeat testing within a week	145	70.5
Fetal biophysical profile assessment	30	14.5
Transport	7	3.4
Total	206	100

Table II. Recommended management for intrapartum fetal heart rate transmissions (total number = 217)

<i>Management</i>	<i>No.</i>	<i>%</i>
Observation	132	61
Delivery	38	17.5
Fetal scalp blood sampling	16	7.3
Medical treatment	25	11.5
Transport	6	2.7
Total*	217	100

*Twenty-eight tracings were excluded from the initial number of 245 because the interpretations were not recorded.

stances of each case, our recommendations were: observation, delivery, fetal acid-base assessment (fetal scalp blood sampling), medical treatment (positional changes, oxygen administration, discontinuation of oxytocin, etc.), or maternal transport (in the presence of prematurity or other complications).

After delivery, follow-up information of maternal and neonatal outcome was requested from the regional hospital. All information obtained from the patient's history, the fetal heart rate tracing, recommended management, and follow-up is coded and entered into a computer.

Results

From January 1, 1983, to June 30, 1985, a total of 511 fetal heart rate tracings were received through the FACTS system. Of these, 206 (40.3%) were antepartum, 245 (48%) intrapartum, and 60 (11.7%) were transmitted for educational purposes. These 60 were eliminated from further analysis.

Of the 206 antepartum fetal heart rate tracings, 188 (91.3%) were nonstress tests and 18 (8.7%) were contraction stress tests. The interpretations for the 188 nonstress tests were: reactive, 115 of 188 (61.2%); non-reactive, 65 of 188 (34.5%); and unsatisfactory, eight of 188 (4.3%). The recommendations for the 65 non-

reactive nonstress tests were: repeat testing, 22 of 65 (34%); delivery, 16 of 65 (24.6%); further evaluation with fetal biophysical profile assessment, 20 of 65 (30.7%); and maternal transport, seven of 65 (10.7%). The interpretations of the 18 contraction stress tests were: negative, eight of 18 (44.4%); positive, five of 18 (27.8%); and suspicious, five of 18 (27.8%). The uterine activity pattern in 10 of the 18 contraction stress tests (55.6%) was normal, in four (22.2%) it was difficult to determine, and in the remaining four (22.2%) there was hyperstimulation. The recommendation for the positive contraction stress tests was delivery in all five cases; delivery was also recommended in three of the five suspicious contraction stress tests and fetal biophysical profile assessment was proposed in the remaining two of the five suspicious contraction stress tests.

The overall recommendations for a total of 206 antepartum fetal heart rate tracings are illustrated in Table I. Of the 206 antepartum fetal heart rate consultations follow-up information was obtained in 70 cases. In 68 instances (97.2%) the Apgar scores were good (5-minute Apgar score $\geq$ 7) and in two (2.8%) the Apgar scores were low (5-minute Apgar scores $<$ 7). The recommendation for both cases with low Apgar scores was delivery, while the recommendations for the 68 cases with good Apgar scores were delivery in 17 (25%), repeat testing within a week in 29 (42.6%), fetal biophysical profile assessment in 17 (25%), and maternal transport in five (7.4%).

Of the 245 intrapartum fetal heart rate tracings, 107 (43.6%) were interpreted as normal, 77 (31.5%) suspicious, 22 (9%) threatening, 11 (4.5%) ominous, and in 28 instances (11.4%) the interpretation was not recorded. These 28 fetal heart rate tracings were therefore excluded from further analysis. The recommendations for the 107 normal fetal heart rate tracings were observation in 99 of 107 (92.5%), delivery in one of 107 (0.9%), medical treatment in three of 107 (2.8%), and maternal transport in four of 107 (3.8%). For the 77 suspicious tracings the recommendations were observation in 30 (38.9%), delivery in 19 (24.6%), fetal scalp blood sampling in 10 (12.9%), medical treatment in 17 (22%), and maternal transport in one (1.3%). For the 22 threatening tracings the recommendations were observation in three (13.6%), delivery in nine (40.9%), fetal scalp blood sampling in four (18.1%), medical treatment in five (22.7%), and maternal transport in one (4.5%). For the 11 ominous fetal heart rate tracings the recommendations were delivery in nine (81.8%) and fetal scalp blood sampling in two (18.1%). The overall recommendations for the total of 217 (28 were excluded from the initial 245) intrapartum fetal heart rate tracings are illustrated in Table II. Table III illustrates the relationship between prenatally identified

Table III. Relationship between prenatal high-risk factors and fetal heart rate interpretation in patients requiring intrapartum transmission

Prenatal high-risk factor	No. of cases	Fetal heart rate interpretation							
		Normal		Suspicious		Threatening		Ominous	
		No.	%	No.	%	No.	%	No.	%
Postdates	135	63	46.6	53	39.3	12	9	7	5.1
Intrauterine growth retardation	14	10	71.5	4	28.5	—	—	—	—
Premature rupture of membranes	5	—	—	—	—	4	80	1	20
Premature labor	18	17	94.5	—	—	—	—	1	5.5
No high-risk factor present	45	17	37.7	20	44.5	6	13.3	2	4.5
No interpretation available	28	—	—	—	—	—	—	—	—

high-risk factors and fetal heart tracing interpretation in patients requiring intrapartum transmission. Post-datism was the most frequent prenatal high-risk factor (135 cases), while in 45 cases no prenatal high-risk factor was identified. Premature labor was associated with the highest incidence of normal fetal heart rate tracings (17 of 18 or 94.5%) and cases with premature rupture of the membranes had the highest incidence of threatening (four of five or 80%) and ominous (one of five or 20%) fetal heart rate patterns.

Oxytocin administration (augmentation or induction) was associated with 109 (44.4%) of the total of 245 intrapartum tracings. In these 109 cases there was hyperstimulation in 31 (28.4%) and a normal uterine contraction pattern in 70 (64.2%); the uterine activity pattern was difficult to determine in the remaining eight (7.3%) cases.

Follow-up information was obtained in 112 of the 245 cases that required intrapartum fetal heart rate transmission. Of these 112 cases, 99 (88.4%) had good Apgar scores and 13 (11.6%) had low Apgar scores. The recommendations for the group with good Apgar scores were observation in 68 (68.6%), delivery in 20 (20.2%), fetal scalp blood sampling in one (1%), medical treatment in eight (8%), and maternal transport in two (2%). The recommendations for the group with low Apgar scores were delivery in 10 (76.9%), fetal scalp blood sampling in one (7.6%), medical treatment in one (7.6%), and observation in one (7.6%).

Comment

The use of the FACTS system, which provides a continuous hard-copy communication between the primary community hospitals and the University of Connecticut Health Center, has been in effect since January 1, 1983. The reported results of our experience during the first 2½ years of its use are very interesting in many respects.

Sixty fetal heart rate tracings, transmitted for education purposes, were excluded from the analysis. Therefore the analysis was confirmed to 206 antepartum and 245 intrapartum fetal heart rate tracings, which were transmitted because the primary obstetric health care provider had serious concerns about the condition of the fetus. Subsequently 28 intrapartum tracings were also excluded because the interpretations were not recorded. It is unknown what the management might have been in these cases without a consultation from our regional center. In the great majority of cases the fetal heart rate tracings, according to our interpretations, were compatible with fetal well-being, and therefore conservative (expectant) management was recommended. Sixty-one percent of the transmitted antepartum fetal heart rate tracings were reactive and expectant management was recommended in all these cases. Similarly, the most frequent interpretation of the intrapartum fetal heart rate tracings (43.6%) was compatible with fetal well-being, and conservative management was recommended in 99% of these cases. We recommended intervention, in terms of prompt delivery, in only 11.6% of the transmitted antepartum fetal heart rate evaluations (Table I) and in 17.5% of the cases transmitted during the intrapartum period (Table II). Retrospectively these recommendations were proved to be appropriate since prompt delivery was advised in both antepartum cases with low Apgar scores and in 10 of 13 intrapartum cases with low Apgar scores. The recommended intervention rate (usually by cesarean section) of 17.5% in the intrapartum cases is very low if one considers the national average cesarean section rate of 17.9% in 1981.⁵ According to the interpretation of the regional care providers, apparently normal fetal heart rate tracings were not transmitted, but only nonreassuring tracings; it would appear therefore that the FACTS system had a major impact in reducing the intervention rate in the regional

hospitals. The low intervention rate combined with the recognition of the need for prompt delivery in the great majority of cases with low Apgar scores is a testimony for the efficacy of the system in improving regional perinatal care.*

Further analysis of the results, according to the presence or absence of prenatal high-risk factors in patients who were delivered in smaller community hospitals and had nonreassuring intrapartum fetal heart rate patterns revealed that the majority (135 of 245 or 55.1%) were postdates. There was no difference in the incidence of abnormal fetal heart rate patterns between patients with postdates and patients with no prenatal high-risk factors (Table III). Premature labor tracings transmitted had the higher incidence of normality (17 of 18 or 94.5%), while tracings transmitted from patients with premature rupture of the membranes had the highest incidence of abnormal fetal heart rate patterns (80% threatening, 20% ominous). The observation of a high incidence of abnormal fetal heart rate tracings in the presence of premature rupture of the membranes may not be pertinent because of the small number of patients with ruptured membranes (five cases).

According to these data, in the region the most frequent problem associated with nonreassuring intrapartum fetal heart rate tracings is postdatism. The efforts therefore in the future should and will be directed to further education of the obstetric staff of these hospitals (physicians and nurses) in managing postdate pregnancy, as well as in fetal heart rate interpretation. Since 28.4% of the intrapartum tracings had evidence of uterine hyperstimulation, the efforts should also be directed toward educating the regional hospitals in judicious use of oxytocin and formulating low-dose protocols.

The FACTS system has also helped us to identify those high-risk patients who need care in a tertiary center for further evaluation and treatment. A total of 13 (2.8%) such cases among the 451 transmission con-

sultations were identified, and the patients were transferred to the University of Connecticut Health Center for further evaluation and treatment. In cases of a requested maternal transport, the regional hospitals are encouraged to use the FACTS system to transmit the fetal heart rate tracing before the transfer, especially in cases where the condition of the fetus may be in question. If it is felt that the fetal heart rate tracing indicates fetal jeopardy, prompt delivery at the primary community hospital is recommended. In such cases, the neonatal team at the health center is notified and arrives at the regional hospital as soon as possible in order to provide expert resuscitation and immediate care of the potentially sick neonate.

It is also our opinion that the FACTS system has a great educational potential since it provides the opportunity to smaller community hospitals (physicians and nurses) for an immediate consultation and educational discussion.

In summary, it is our opinion, based on the present reported experience, that the use of the FACTS system improves the quality of perinatal care in the regional hospitals, it helps in the identification of those high-risk patients who may benefit from maternal transport, and it is a tool that could be effectively used to discover specific areas toward which educational efforts should be directed in the smaller community hospitals.

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Diabetes in pregnancy: Evaluating self-monitoring performance and glycemic control with memory-based reflectance meters

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Reflectance meters modified by the addition of a memory microchip that stored glucose values were used to measure the difference between self-reported and actual blood glucose data in 34 pregnant diabetic women. Overall two thirds of the patients reported values that were significantly lower and less variable than those recorded in the memory meters. (AM J OBSTET GYNECOL 1986;155:635-7.)

Key words: Diabetes in pregnancy, self-monitoring, verified blood glucose data

Evidence that the quality of glycemic control among pregnant diabetic women relates to fetal and maternal outcome is currently derived from patient self-reported blood glucose data.¹ The present study, with the use of reflectance meters modified with memory chips, permitted the novel approach of recording results from each capillary blood glucose test without the patient's knowledge. This enabled characterization of the reliability and accuracy of self-monitored blood glucose during pregnancy, a time at which high motivation could be anticipated to produce optimal compliance.

Material and method

Thirty-four women (mean age 30 ± 5 years) with gestational ($N = 21$) or pregestational ($N = 13$) diabetes were evaluated between the sixteenth and thirty-ninth gestational weeks (mean 27 ± 10 weeks). All patients were married and high school graduates, with 50% ($N = 17$) having attended college. Past obstetric history indicated: (1) 34% with spontaneous abortions, (2) 11% with premature neonates, (3) 17% with pregnancy-induced hypertension, (4) 23% with a previous macrosomic infant, and (5) 17% with a history of stillbirths.

Study participants were treated with multiple injections of mixed insulins or diet alone. All women were taught and evaluated for self-monitoring techniques with the use of a Glucometer Reflectance Photometer

modified with a memory microchip capable of storing 440 glucose values with the corresponding time and date.² The women were asked to maintain careful logbook records but were not told of the memory modification. During a 14-day study period the women were asked to measure their capillary blood glucose values at least seven times daily. At weekly clinic visits, the accuracy of the meters were checked against laboratory assays of matched venous samples. A previously described scoring system to measure patient performance was used to analyze the data.²

Results

Performance as measured by comparison between memory meter and logbook records indicated that 33 of the 34 patients (97%) omitted from their logbook glucose values that were recorded in their memory reflectance meters. This resulted in an average underreporting score of $23\% \pm 18\%$, or approximately one recording dropped for every five actual determinations. One third of the patients exceeded the underreporting average, resulting in a score of 46% or one value deleted for every two recordings.

Twenty-five patients (74%) added glucose values that were not determined by the reflectance meter, resulting in an average overreporting score of $23\% \pm 31\%$. Overreporting ranged from 0% to 88% with eight patients found to have added at least one unverified glucose value for each meter-determined value. An examination of reliability revealed a significant correlation between overreporting and underreporting ($r = 0.44$, $p = 0.01$).

Thirteen patients achieved 100% precision. This, however, was not shown to be related to reliability, since three of these individuals proved to have among the highest combined overreporting and underreporting scores. The mean precision score for all subjects was 88%, ranging from 53% to 100%.

A comparison of mean memory values with logbook

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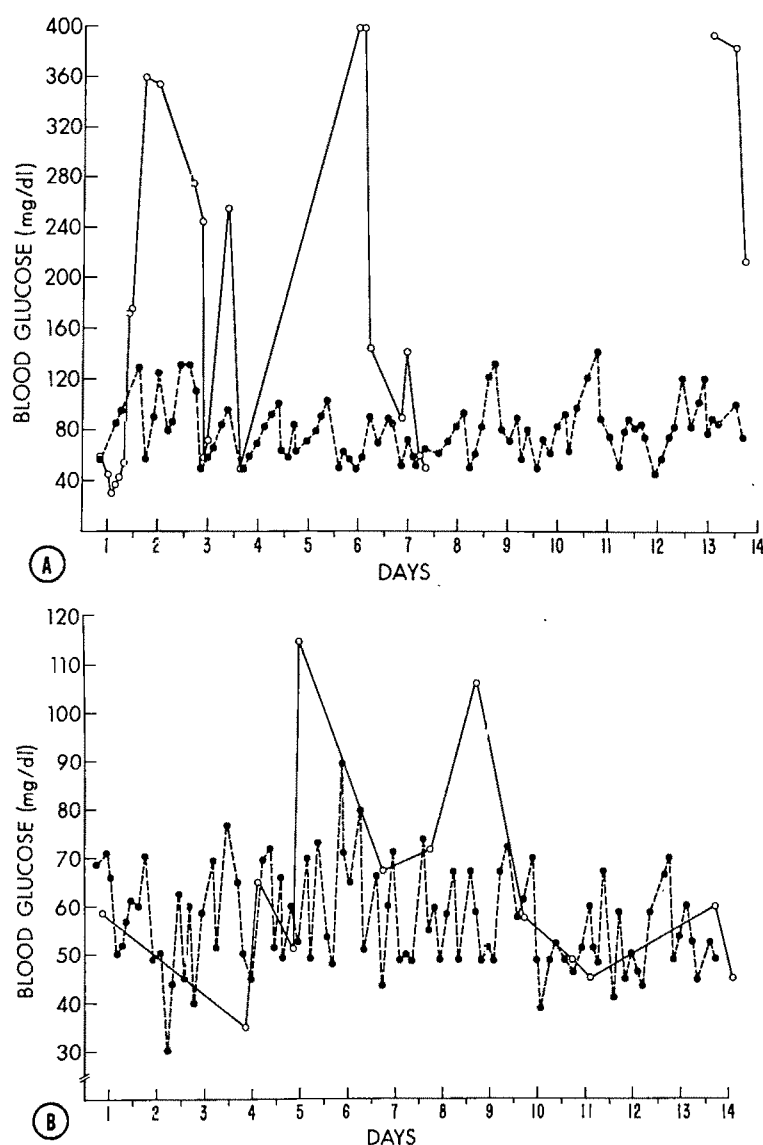


Fig. 1. Representations of individual patterns of self-monitoring performance. The *closed circles with dashed lines* represent the logbook glucose values. *Open circles with solid lines* represent memory meter glucose values.

values revealed a 10 mg/dl excess (103 ± 25 versus 93 ± 20 mg/dl), which was found to be significant ($p = 0.05$). A significant difference ($p = 0.0004$) was also found between the mean coefficient of variation for the logbook records ($31\% \pm 12\%$) and the memory meter ($39\% \pm 14\%$). Logbook data consistently produced both less variability and a narrower range than memory data. The relationship between glycemic control as recorded in the meter and performance as determined by overreporting and underreporting scores was not significant. Further, no significant relationship was uncovered when hemoglobin A_{1c} or random laboratory blood glucose values were compared with performance.

A review of the relationship between patient characteristics and performance was undertaken. Type of diabetes was not found to be related to performance. Underreporting for gestational diabetic patients averaged 22% compared with 25% for pregestational diabetic patients. Overreporting averaged 21% for the gestational diabetic patients and 28% for the pregestational diabetic patients. Additionally, no significant relationship between performance and previous outcome of pregnancy or between performance and obesity was uncovered. Since patients were admitted to the study at different gestational weeks, we determined whether this influenced accuracy or reliability. No significant relationship between gestational week

and overreporting, underreporting, or precision was found, nor was the difference in therapy (insulin versus diet) related to overall performance.

The self-monitoring performance of the individual patients revealed several patterns. Patient No. 13 typified both underreporting and overreporting (Fig. 1, A). She completed 26 tests, reported 15 in the logbook, and added 74 unverified values to her records, resulting in a logbook average of 83 ± 22 mg/dl versus a memory mean of 164 ± 130 mg/dl. This pattern was uncovered in 6% of the women. Forty percent of the patients actually tested on fewer days than recorded in the logbooks, resulting in a difference in mean values as well as in coefficient of variation (typified by Patient No. 14 in Fig. 1, B). A small percentage (9%) repeatedly tested at the same time each day and then selected the lowest glucose value to report in the logbook. Two patients stopped testing after several days but continued to report the same values, resulting in no difference between logbook and meter values.

Comment

The findings show that performance was unpredictable and may be unreliable among presumably well-motivated women. For 80% of the subjects significant differences exist between mean blood glucose and co-

efficient of variation when memory meter and logbook values are compared with a tendency toward a narrowing of the range of reported glucose values. This resulted in a masking of acute and chronic hyperglycemic episodes. Thus suggestions for alterations in insulin and diet regimen, generally based on the patients' self-reported glucose data, particularly data related to excursions from the normal range, could be unrelated to the patients' actual clinical status.

If the hallmark in diabetes care has been a partnership between patient and physician, reflected in the exchange of important clinical information, then this exchange has to be reliable and accurate. This, in turn, will enhance our ability not only to characterize and verify the level and quality of glycemic control during pregnancy but also to assess the contribution of nonglycemic metabolic factors to maternal and fetal outcome.

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Efficacy of mathematical methods for ultrasound examinations in diabetic pregnancies

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The efficacy of a new principle for evaluation of fetal growth has been compared to standard methods in a population of pregnant diabetic women. Methods for projecting fetal weight at birth and determining the deviation of growth from an expected fetal weight increase based on our growth model provided significant information for the detection of fetal growth abnormalities 3 weeks (mean) before delivery. (Large for gestational age: sensitivity, 81%; specificity, 85%. Small for gestational age: sensitivity, 100%; specificity, 98%.) With the exception of two models for estimation of fetal weight based on ultrasound measurements of the fetal head and abdomen only, none of the standard methods gave results that were significantly better than the use of the biparietal diameter against a population standard. The new methods are expected to provide valuable information for the treatment of fetal growth disorders. (AM J OBSTET GYNECOL 1986;155:638-44.)

Key words: Diabetes, fetal growth, ultrasound, mathematical methods.

Maternal diabetes is a major risk factor in pregnancy. Thus at the Center for Pregnant Diabetics at the Rigshospital, Copenhagen, a perinatal mortality rate of 59.2 was observed for 490 pregnancies from 1970 to 1975,¹ whereas the national perinatal mortality rate for Denmark was 14.9 for the years 1971 to 1975.² This indicates a fourfold risk of fetal or neonatal death in the diabetic pregnancy even though the principles of management included centralization, hospitalization, and ultrasound cephalometry at least three times from 32 weeks of gestational age to term for pregnant women with diabetes mellitus.¹

Indigent pregnant diabetic patients attending the Diabetic Clinic at the Harris County charity hospital, Jefferson-Davis Hospital, for prenatal care are not routinely hospitalized, and in fact a major portion of them are seen only in the last couple of weeks before delivery.

The objective of the present study was to evaluate the efficacy of different ultrasound methods in predicting anomalous fetal growth (large for gestational age or small for gestational age) in a population with a high prevalence of fetal growth problems.

Three different principles were used to extract information about fetal growth: (1) Ultrasound measurements or simple combinations of ultrasound measurements were compared to ultrasound standards for

normal growth. (2) Estimates of fetal weights were compared to Brenner et al.'s³ standard of fetal growth for the United States. (3) Ultrasound measurements before 30 weeks' menstrual age were used to project fetal growth after 30 weeks. Comparisons with measurements after 30 weeks were made to decide growth deviation and to make birth weight projections.

Material and methods

The study included 55 pregnancies from a predominantly Latin-American and black population who fulfilled the criteria for acceptance in the present study. The study included the time period from March 1983 to March 1985, during which period a total of 325 diabetic pregnant women had their antenatal care at the Diabetic Clinic at Jefferson-Davis Hospital. The patient material was classified in the following way: Group 1a—study group. Patients who were registered at the clinic in early pregnancy (before 30 weeks) and who had ultrasound examinations in our laboratory both before and after 30 weeks ($n = 55$). Group 1b—patients who were registered at the clinic in early pregnancy who had ultrasound examinations in other or different laboratories and therefore did not satisfy the criteria for group 1a ($n = 68$). Group 2—neglecters.⁴ Patients who were registered at the clinic after 30 weeks or patients who missed two or more appointments ($n = 202$).

The patients (group 1a) had 213 ultrasound examinations performed between 11 and 40 weeks of pregnancy; 99 examinations (average, 1.8 per patient; range, one to four) examinations were performed before 30 weeks and 114 (average, 2.1 per patient; range

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one to four) examinations were performed after 30 weeks' menstrual age. Gestational age was determined from the last menstrual period. Ultrasonographic determination of gestational age was used for patients who did not remember their last menstrual period, had irregular periods, and/or had a discrepancy of >14 days between gestational age based on last menstrual period and gestational age based on ultrasound.

Four different real-time linear-array or combined linear-array/sector ultrasound scanners were used for the examinations. The measurements, which were the biparietal diameter (BPD), the occipitofrontal diameter (OFD), the abdominal longest diameter (ALD), the abdominal shortest diameter (ASD), and femur length (FL), were taken directly from the screen with electronic callipers, and each measurement was repeated at least once on a new image before it was accepted. Measurements of the fetal head were taken with mid-point-midpoint technique whereas the abdominal diameters were measured at the outer contour.³

Large for gestational age was defined as birth weight at or above the ninetieth percentile, whereas small for gestational age was defined as birth weight at or below the tenth percentile according to the standard by Brenner et al.³

Parameters and simple parameter combinations.

The measured diameters were used to calculate the head cube [$A = (\text{BPD} \times \text{OFD})^{1.5}$], the abdomen cube [$B = (\text{ALD} \times \text{ASD})^{1.5}$], the head circumference [$\text{HC} = (\text{BPD}/2 + \text{OFD}/2) \times 3.14$] and the abdominal circumference [$\text{AC} = (\text{ALD}/2 + \text{ASD}/2) \times 3.14$]. The following parameter-combinations were tested for their efficacy to detect large for gestational age growth: A/B ratio,⁵ ASD - BPD,⁶ FL/AC ratio,⁷ ASD/FL ratio, and ASD/BPD ratio.⁸ Measurements of the BPD were compared to a population standard.³

Equations used to estimate fetal weight. The following equations for estimation of fetal weight were tested for their accuracy in separating large for gestational age and small for gestational age fetuses from those who were appropriate for gestational age:

BPD, HC, AC, and FL (Hadlock et al.¹⁰)

A, B, and MA (Rossavik¹¹):

$$\text{Wt} = 0.545A(D - 37)^{0.00072(D - 37)} + 1.55B(D - 47)^{-0.000011(D - 47)} \quad (1)$$

where D is menstrual age (MA) in days and Dmax used in equation equals 260.

BPD and AC (Shepard et al.¹²)

BPD and AC (Warsof et al.¹³)

Use of fetal growth rate before 30 weeks to predict individual growth curves for comparison with observations after 30 weeks. Basic growth equation used (the Rossavik growth model):

$$P = ct^{k+s} \quad (2)$$

where P = parameter, t = time calculated from the zero-point, c is a coefficient mainly indicating growth rate in the first two trimesters, coefficient s reflects changes in growth rate in the last trimester, and k is a coefficient with value related to the dimension of the parameter.^{11, 14}

Predicted growth curves. Geometric mean of head (BPD and OFD) and abdomen measurements (ALD and ASD) obtained before 30 weeks' menstrual age were used to determine the slope of the linear phase of growth and to evaluate the zero-point or starting point of growth as recommended for the Rossavik model.¹⁴ Coefficients c of the growth equation (equation 2) were determined from the slopes of the linear growth models. A high correlation has been found between the coefficients c and s.¹¹ The predicted s can therefore be calculated from the value of c. The difference between s and predicted s, the s-residual, represents the part of s which is not correlated to c. Therefore the s-residual reflects changes in growth rate observed after 30 weeks of pregnancy. The following equations were used to determine the predicted s-coefficients¹¹:

For the A growth curve: $s = -224.6c - 0.00094$ (3)

For the B growth curve: $s = -33.0c + 0.000182$ (4)

Actual growth curves. Values for A and B were calculated and interpolated to 170 days (24.3 weeks) of pregnancy for each fetus from data obtained before 30 weeks of pregnancy. The reference point, 24.3 weeks (5.7 weeks from 30 weeks' menstrual age), was chosen because measurement errors will have an increased impact on the observed coefficients if the time interval between this point and the examination after 30 weeks is too short. Observed values for A and B after 30 weeks of pregnancy (A_1 and B_1) and interpolated values for A and B at 24.3 weeks of pregnancy (A_2 and B_2) were used in the following equation (derived from equation 2) to calculate actual s-coefficients¹¹:

$$s = \frac{\ln P_1 - \ln P_2 - k(\ln t_1 - \ln t_2)}{t_1 \times \ln t_1 - t_2 \times \ln t_2} \quad (5)$$

where for the fetal head $P_1 = A_1$, $P_2 = A_2$, $k = 4$, and $t = D - 37$ and for the fetal abdomen $P_1 = B_1$, $P_2 = B_2$, $k = 3.45$, and $t = D - 47$.

Average longitudinal growth curves. Previous studies have indicated that normal individual curves for fetal weight parallel the course of an average longitudinal weight curve after 30 weeks of pregnancy.¹¹ This principle was used to improve the accuracy of weight projections and to detect growth deviation occurring after 30 weeks. Thus an average longitudinal fetal weight curve was derived with use of equation 1 and the average longitudinal growth curves for A and B¹¹:

Table I. White classification and outcome for the different groups of pregnant diabetics

	Group 1—patients with good prenatal care			Group 2—neglecters (n = 202)
	1a (n = 55)	1b (n = 68)	Total (n = 123)	
White classification				
A patients (%)	11 (20)	18 (27)	29 (24)	110 (54)
B patients (%)	35 (64)	37 (54)	72 (59)	75 (38)
C-D patients (%)	9 (16)	13 (19)	22 (18)	17 (8)
Outcome at birth				
Birth weight (gm)	1430-4660	227-4870	227-4870	1389-5740
MA at birth (wk)	33.6-41.3	18.9-43.3	18.9-43.3	32.6-45.7
Prevalence of LGA (%)	38.2	19.1	27.6	32.7
Prevalence of SGA (%)	10.9	10.2	10.6	4.4
Perinatal mortality per 1000				
MA >16 wk	0.0	102.9	56.9	0.0
Birth weight >1000 gm	0.0	29.4	16.3	0.0

LGA = Large for gestational age. SGA = Small for gestational age. MA = Menstrual age.

$$A = 0.00000309(D - 37)^4 - 0.00161(D - 37) \quad (6)$$

$$B = 0.0000224(D - 47)^{3.45} - 0.000585(D - 47) \quad (7)$$

Projected birth weights

Projection 1. The difference between actual weight estimate and the prediction given by the average longitudinal growth curve was added to the average fetal weight given by the longitudinal weight curve at 265 days' menstrual age.

EXAMPLE 1. Values at reference point 24.3 weeks (170 days' menstrual age) were: $A_2 = 315 \text{ cm}^3$, $B_2 = 217 \text{ cm}^3$. Examination at 211 days' menstrual age: $A_1 = 700 \text{ cm}^3$, $B_1 = 647 \text{ cm}^3$. Estimated weight: 1722 gm. Average weight at 211 days (equations 6, 7, and 1): 1617 gm. Difference between estimated and average weight: 105 gm. Average weight at 265 days' menstrual age (equations 6, 7, and 1): 3495 gm. Projected weight at 265 days' menstrual age: 3495 gm + 105 gm = 3600 gm.

Projection 2. Fetal weight at 265 days' menstrual age predicted by means of actual growth curves (equations 1, 2, and 5).

EXAMPLE 2. Head cube growth curve (equation 5), $s = -0.00112$. Abdominal cube growth curve (equation 5), $s = 0.00041$. Projected A at 265 days' menstrual age (equation 2): 1411 cm^3 . Projected B at 265 days' menstrual age (equation 2): 1985 cm^3 . Projected weight at 265 days' menstrual age (equation 1): 4870 gm.

Projection 3. Fetal weight at 265 days' menstrual age projected from early pregnancy data (predicted growth curves based on slopes of linear growth phases, equations 3, 4, 2, and 1).

EXAMPLE 3. Slope of the linear growth phase calculated from reference value at 24.3 weeks' menstrual age; fetal head: [slope = $(A_2)^{1/3}/(D - 37)$]: 0.051. Coefficient $c = (0.051)^4 \times 0.4426 - 0.00000018 =$

0.0000029. Coefficient s (equation 3) = -0.00158 . Projected A at 265 days' menstrual age (equation 2) = 1090 cm^3 . Fetal abdomen, slope: [Slope = $(B_2)^{1/3}/(D - 47)$]: 0.049. Coefficient $c = (0.049)^3 \times 0.2188 - 0.0000072 = 0.0000183$. Coefficient s (equation 4) = -0.00042 . Projected B at 265 days' menstrual age (equation 2) = 1303 cm^3 .

Projection 3, weight (equation 1): 3400 gm.

Method of discrimination between growth patterns. The following procedure, which represents a method to discriminate between the different growth patterns for large for gestational age, appropriate for gestational age, and small for gestational age fetuses,¹¹ was used to project birth weight at examinations performed after 30 weeks of pregnancy: If projection 2 indicated a birth weight of ≥ 200 gm lower than that indicated by projection 1, growth deceleration was considered to have occurred and projection 2 was chosen (by the computer program*) to represent projected birth weight for examinations performed after 30 weeks. If the difference between these two projections was less than 200 gm or if projection 2 was higher than projection 1, the average of projection 1 and projection 2 was used as the projected birth weight.

EXAMPLE 4. Projection 1: 3600 gm, projection 2: 4870 gm. "Projected weight at birth," average of projected weights at reference point 265 days' menstrual age: 4235 gm. This patient was delivered at 283 days' menstrual age. Birth weight was 3970 gm. Error: 265 gm, percent deviation: 6.7% (the difference in time between reference point 265 days' menstrual age and gestational age at delivery has not been taken into account for infants born after 36 weeks' menstrual age).

*The calculations were automated with use of the computer programs Prenatal Care Ultrasound Reports 1 and 2 (copyright by Rossavik). Templates for SuperCalc 3 (copyright by Sorcim Corporation).

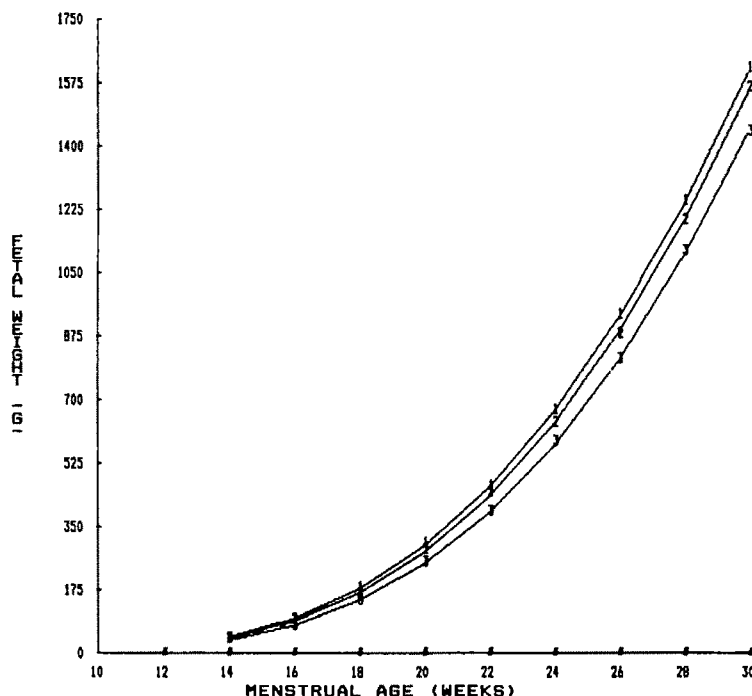


Fig. 1. Fetal growth curves (weight) in diabetic pregnancies until 30 weeks' menstrual age. 1 = Mean longitudinal curve for large for gestational age newborn infants. 2 = Mean longitudinal curve for appropriate for gestational age newborn infants. 3 = Mean longitudinal curve for small for gestational age newborn infants.

Limits for projected weights "at birth": Large for gestational age = projected weight >3750 gm, small for gestational age = projected weight <2750 gm.

The three different parameters used to detect growth deviation were evaluated in the following ways (empiric limits):

1. *Head cube A.* The s-residual was the calculated difference between the s-coefficient in the predicted function (equation 3) and the actual function (equation 5).

EXAMPLE 5. S-residual for the head cube growth curve: s (example 2) - predicted s (example 3) = $-0.00112 - (-0.00158) = 0.00046$. Limit for deviation: ± 0.00030 .

2. *Abdominal cube B.* The s-residual was the calculated difference between the s-coefficient in the predicted function (equation 4) and the actual function (equation 5).

EXAMPLE 6. S-residual for the abdominal cube growth curve: s (example 2) - predicted s (example 3) = $0.00041 - (-0.00042) = 0.00083$. Limit for deviation: ± 0.00045 .

3. *Deviation in projected birth weights.* Projected birth weight based on projection 3 was subtracted from projected birth weight based on projection 1 and projection 2. If the difference between these two projections was ≥ 500 gm, the fetus was considered to be large for gestational age (positive deviation) or small for gestational age (negative deviation).

EXAMPLE 7. Projected birth weight minus projection = 4235 gm - 3400 gm = 835 gm.

Ultrasound observations for the different methods were compared to outcome at delivery with use of a two-by-two table; χ^2 test with Yates' correction was used to find statistical significance of observed differences between positive and negative tests in two different groups of outcome at delivery.

Results

Outcomes for the three different groups (groups 1a, 1b, and 2) are shown in Table I. Although the study group (group 1a) had the highest prevalence of both large and small for gestational age fetuses, no fetal loss occurred in this group. The total fetal loss in group 1 was seven fetuses of whom four weighed <500 gm and five were delivered before 30 weeks and would therefore not be included in group 1a. The two stillbirths after 30 weeks in group 1b were small for gestational age fetuses and are included in this subgroup. Because of the higher prevalence of fetuses large for gestational age in group 1a than in group 1b, it seems reasonable to conclude that clinical preselection to some extent occurred before the patients were given appointments for ultrasound studies. This preselection has probably been less effective for small for gestational age fetuses, which may also be the reason why two stillbirths occurred in group 1b. The prevalence of large for ges-

Table II. Group 1a—basic statistics for fetal growth and observations at birth

	LGA (n = 21)	AGA (n = 28)	SGA (n = 6)
Outcome at birth:			
Birth weight, mean (gm)	4134	3158	2208
Birth weight, range (gm)	3530-4660	2430-3730	1430-2608
Menstrual age at birth, mean (wk)	38.9	38.9	37.4
Menstrual age at birth, range (wk)	37.0-40.9	35.9-41.3	33.6-39.0
Fetal head			
S-residual, mean	0.00026	0.00004	-0.00017
S-residual, 1 SD	0.00023	0.00021	0.00028
Fetal abdomen			
S-residual, mean	0.00044	-0.00003	-0.00074
S-residual, 1 SD	0.00039	0.00034	0.00022
Projected weight at birth			
Percent deviation, mean	-2.0	3.0	-0.7
Percent deviation, 1 SD	12.4	12.9	6.2
Apgar score at 1 min			
Mean	7.95	7.81	6.83
1 SD	1.23	1.52	1.72
Interval, last examination to birth			
Mean (days)	22	22	16
Range (days)	1-52	3-59	2-38

tational age fetuses in the neglecter group was higher than in group 1. On the other side was the very low prevalence of small for gestational age fetuses among neglecters—only nine small for gestational age fetuses instead of the 21 which would have been found if the prevalence of small for gestational age fetuses in group 2 had been the same as in group 1. The high frequency of Class A diabetes in group 2 may be the explanation for the decreased prevalence of small for gestational age fetuses (Table I). It is, however, also possible that growth retardation in the neglecter group frequently ends in fetal loss and is therefore not registered at the outpatient clinic.

The mean growth curves for the period before 30 weeks' menstrual age for fetuses who later became large, appropriate, or small for gestational age newborn infants are given in Fig. 1. The maximum difference between the curves is not more than 150 gm, which indicates similar growth rates before 30 weeks of pregnancy for all three subsets of the study group because the difference between the tenth and ninetieth percentiles according to the applied standard³ is >1000 gm at 30 weeks. Basic statistics for observed fetal growth and observations at birth are given in Table II. The s-re-

sidual indicates the degree of growth deviation occurring after 30 weeks. For the fetal abdomen the mean s-residual of the small for gestational age group was approximately 2 SD below that of the appropriate for gestational age group, while the mean s-residual of large for gestational age fetuses was between 1 and 2 SD above that of appropriate for gestational age fetuses. For the fetal head these differences were only 1 SD for both large for gestational age and small for gestational age fetuses. Two newborn infants were omitted from the calculations of differences between projected and real weights at birth because of preterm delivery before 36 weeks; no other adjustments for relations between projected weights, real birth weights, or gestational age at birth were performed. Nevertheless, mean random errors were within $\pm 3\%$, and 1 SD was <13% for each of the three subsets; large, appropriate and small for gestational age fetuses even though their mean weights at birth were 4134, 3158, and 2208 gm, respectively, and the examinations were performed several weeks before delivery (Table II).

The efficacy of different ultrasound methods in predicting size that is large for gestational age in newborn infants is given in Table III. Since the study group had a high prevalence of large for gestational age infants (38%), the predictive values of positive tests for all methods based on more than one examination and for estimated weights were between 75% and 88%. It seems appropriate to conclude that the results obtained for two of the tests, projection of birth weight and weight deviation, promise clinical usefulness also in a population with a lower prevalence of growth acceleration. With the exception made for weight estimation with use of either the Shepard or the Rossavik equation, none of the other methods gave results that supported their use in the diagnosis of large for gestational age birth weights.

The results for projection of birth weight and determination of growth deviation in the prediction of small for gestational age infants were even better than those obtained for large for gestational age infants (Table IV); the group is, however, small. All small for gestational age fetuses were detected by weight projection, weight deviation, and the s-residual of the abdomen cube. The latter test had, however, too many false positive results that gave a specificity of 90%, which is too low for a population with a prevalence of small for gestational age births of only 10%. The results obtained by use of conventional weight estimations were no better than that obtained with BPD versus a population standard.

Comment

Scandinavian centers for pregnant diabetics^{1, 15} have advocated hospitalization including weekly ultrasound

Table III. Efficacy of different ultrasound methods to detect LGA fetuses in diabetic pregnancies

	Sensitivity (%)	Specificity (%)	χ^2	<i>p</i>
Methods based on more than one examination				
Weight projection	81	85	21.08	<0.001
Weight deviation	67	94	20.40	<0.001
S-residual abdomen	48	94	10.92	<0.001
S-residual head	43	91	6.93	<0.01
Estimated weights versus Brenner et. al.'s standard				
Hadlock's equation	45	97	10.94	<0.001
Rossavik's equation	67	91	17.72	<0.001
Shepard's equation	67	91	17.72	<0.001
Warsof's equation	38	97	9.29	<0.005
Other methods based on one examination				
A/B ratio	43	91	6.93	<0.01
ASD-BPD	62	79	7.87	<0.01
FL/AC ratio	45	87	5.01	<0.05
ASD/FL ratio	85	61	8.81	<0.005
ASD/BPD ratio	81	47	3.27	NS
BPD	48	91	8.78	<0.005

examinations from a gestational age of 32 to 33 weeks until delivery. Our regimen does not include hospitalization except for special situations in which the patients' glucose levels are out of control or when intrauterine growth retardation is suspected. Nevertheless, the results obtained for our pregnant diabetic population, which includes a neglecter group of approximately 60% of the total population, are fully comparable with those obtained at centers providing hospital care of long duration and strict metabolic control. Thus Jervell et al.¹⁵ had a 10.7% occurrence of large for gestational age, defined as birth weight above the 97.5th percentile of a Norwegian standard,¹⁶ whereas that definition gave a prevalence of 5.5% large for gestational age in our total population (18/325).

More than 50% of our observed perinatal mortality occurred in fetuses weighing <500 gm. Apart from rigorous treatment of pregnant diabetic patients from very early pregnancy, no method seems available to improve this situation. Overt and hidden pregnancy loss after 30 weeks appeared, however, to occur mainly from intrauterine growth retardation. Since the outcome of this complication depends on early and precise diagnosis, better ultrasound methods for its early detection are needed. Ott et al.¹⁷ analyzed the efficacy of eight fetal weight formulas in 1099 fetuses who underwent ultrasound examinations before delivery and found for all formulas that weights of small for gestational age infants were systematically overestimated, whereas the weights of large for gestational age infants were systematically underestimated. Observing an overall standard deviation of 13.48% for the best equation for measurements performed within 72 hours before delivery, they suggested that future efforts in the development of an accurate method of estimating fetal weight should be directed in separating growth-re-

Table IV. Efficacy of various ultrasound methods to detect SGA fetuses in diabetic pregnancies

	Sensitivity (%)	Specificity (%)	Accuracy (%)
Methods based on more than one examination			
Weight projection	100	98	98
Weight deviation	100	94	95
S-residual abdomen	100	90	91
S-residual head	33	100	93
Estimated weights versus Brenner et. al.'s standard			
Hadlock's equation	67	100	96
Rossavik's equation	67	100	96
Shepard's equation	67	100	96
Warsof's equation	67	98	95
Other method based on one examination			
BPD	67	96	93

tarded and growth-accelerated fetuses from those that are average for gestational age.

A prerequisite for projections of fetal weight to a later date is a weight model that is virtually free from systematic errors in different weight and gestational age groups as well as in growth-retarded and growth-accelerated fetuses. Other prerequisites are small random errors and a growth function that adequately describes and identifies both normal and abnormal fetal growth. Both systematic and random errors for weight projections performed at an average of 3 weeks before delivery (range: 1 to 59 days) in small, large, and appropriate for gestational age newborn infants were less than those observed for weight estimates performed within the last 3 days before delivery.¹⁷ Therefore our method appears to offer a valuable tool for early detection of growth deviation and for evaluation of treatment efficacy in fetal growth disorders.

Weight projection separated large and small for gestational age fetuses clearly from those that were appropriate for gestational age even though the examinations were performed several weeks before delivery. Deviation of projected weights, which uses the same mathematical functions as weight projection, had slightly reduced accuracy when tested against the newborn infant's weight for gestational age. The two methods describe two different parts of the growth process. Thus, while the limits for projection of "weight at birth" are close to the current definitions of intrauterine growth retardation or large for gestational age birth weight, deviations of fetal growth indicate retardation or acceleration of growth rate. The results from both principles may be evaluated in relation to each other, and it is possible that a significant growth deviation is more detrimental to the health of the newborn infant than the actual size-gestational age relationship at birth.

A recommendation of routine use of these methods can, however, not be given unless a larger trial supports the present results in a population with a lower prevalence of growth deviation. The major disadvantage incorporated in our method is that since it is based on a longitudinal evaluation of fetal growth, maximum accuracy can only be obtained if the patient has at least two ultrasound examinations before 30 weeks of pregnancy. At present this limitation restricts the full use of the method to cases considered to be at high risk for fetal growth abnormalities such as patients with hypertension, multiple pregnancies, diabetic pregnancies, and patients with poor obstetric histories.

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Magnetic resonance imaging and ultrasonography in the antenatal evaluation of conjoined twins

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Magnetic resonance imaging and sonography were used as diagnostic aids in the antepartum evaluation of two sets of conjoined twins. Magnetic resonance imaging was performed at 20 weeks' estimated gestational age on thoracopagus/omphalopagus twins and on omphalopagus twins at 20 and 30 weeks. Magnetic resonance imaging is a complementary adjunct to ultrasonography and provides additional anatomically precise clinical data. The advantages of this noninvasive technique include a large diagnostic window allowing total fetal imaging with excellent resolution of tissue composition. Unlike computerized tomographic imaging, there is no associated radiation exposure. The principal disadvantages of magnetic resonance imaging at this time are the cost of the equipment and the lack of real-time imaging capability. (AM J OBSTET GYNECOL 1986;155:645-9.)

Key words: Magnetic resonance imaging, ultrasonography, conjoined twins

The principal diagnostic tool currently employed in the antenatal evaluation of conjoined twins is ultrasonography.¹⁻³ Although preliminary reports have shown the complementary nature of magnetic resonance imaging as an antenatal diagnostic tool with applications in obstetrics,⁴⁻¹⁰ its use in the antenatal evaluation of conjoined twins has not been reported. We present two cases of conjoined twins who were evaluated by both ultrasonographic and magnetic resonance imaging techniques. These two cases demonstrate the strengths and weaknesses of the new diagnostic modality of magnetic resonance imaging.

Material and methods

Based on available laboratory and clinical data, the Institutional Review Board of the University of Texas Health Science Center at Dallas has granted approval for magnetic resonance imaging of pregnant patients who meet certain criteria, including concurrent medical

and/or surgical disease and an abnormal or dead fetus (as determined by sonography). Standard informed consent was obtained in both cases.

Magnetic resonance imaging was performed with a Dasonics 0.35-T (15 MHz) superconductive imaging unit. Serial transaxial, sagittal, and coronal images were obtained. The matrix was 128 by 256 elements, giving a pixel size of 1.7 mm; the section thickness was 7 mm and separation was 3 mm. Spin-echo pulse sequences were used with a pulse repetition time of 500 msec and echo delay of 28 and 56 msec.

Patient 1. This 27-year-old black woman, gravida 2, para 1, presented at 10 weeks' estimated gestational age for routine obstetric care. Sonography performed at 19 weeks revealed omphalopagus twins, appropriate for gestational age, with concordant biparietal diameter measurements. The initial magnetic resonance imaging study at 20 weeks' gestation demonstrated a connection in the upper abdomen of the twins. The mother was placed at bed rest, and serial sonographic evaluations showed concordant fetal growth. The second magnetic resonance imaging study was performed at 30 weeks' EGA, and pertinent findings included both fetuses in the breech presentation, facing each other, and connected by a bridge of tissue. Clearly separate heads, chests and hearts were noted. They were joined in the upper abdomen and shared hepatic tissue, but no major intrahepatic vessels were seen in the tissue bridge (Figs. 1 to 3). Having undergone spontaneous torsion about their abdominal connection, the fetuses were found to be in a vertex/breech presentation at 37 2/7 week's estimated gestational age (Fig. 4). A primary vertical Ce-

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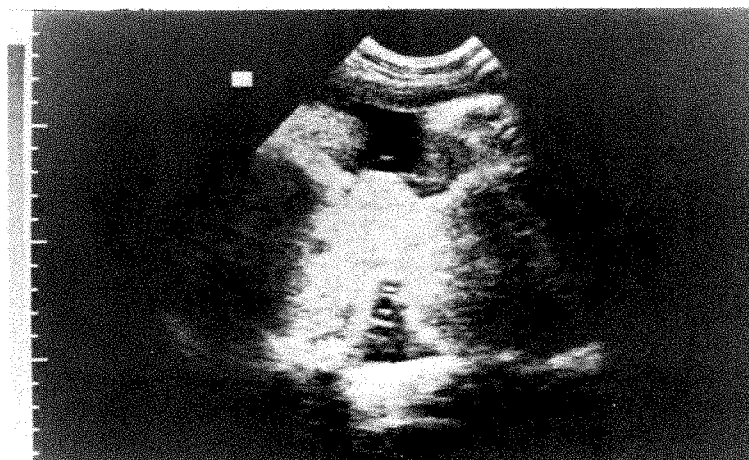


Fig. 1. This sonogram demonstrates the hepatic bridge at 30 weeks' estimated gestational age in the twins of patient 1.



Fig. 2. This coronal image shows the hepatic bridge at 30 weeks.

sarean section was performed with simultaneous delivery of the left vertex and right breech male twins. Their combined weight was 5.27 kilograms and their Apgar scores were 8 and 9 and 8 and 8 at 1 and 5 minutes, respectively. The umbilical cord contained six vessels with a velamentous insertion of the six vessels converging in the midportion of the placenta.

Following delivery, a single umbilical insertion with 5 cm bridge of skin-covered tissue superior to the insertion of the cord was confirmed (Fig. 5). Upper gastrointestinal contrast radiologic studies revealed no bowel-to-bowel anastomoses. The echocardiogram on twin A showed a ventricular septal defect with an overriding aorta separate from the mitral valve, while twin B had no cardiac anomalies. Separation was performed on the night of delivery and confirmed the



Fig. 3. This coronal image shows complete separation of the fetuses in this plane (also at 30 weeks).

magnetic resonance imaging impression of a liver and soft tissue bridge extending from the xiphoid to just below the umbilicus. After the operation, twin A developed early congestive heart failure, which resolved when treated with digoxin and furosemide. He was discharged to his home to await corrective cardiac surgery as necessary. Twin B required ampicillin and gentamicin for postoperative infection and a partial small bowel obstruction that resolved rapidly. He was discharged to his home in satisfactory condition.

Patient 2. A 22-year-old black woman, gravida 3, para 1-0-1-1, presented at 15 weeks' gestation for routine obstetric care. Antenatal ultrasonography performed at 19 weeks because of clinical suspicion of a twin gestation revealed thoracopagus/omphalopagus conjoined twins. One twin had hydrocephaly, and the

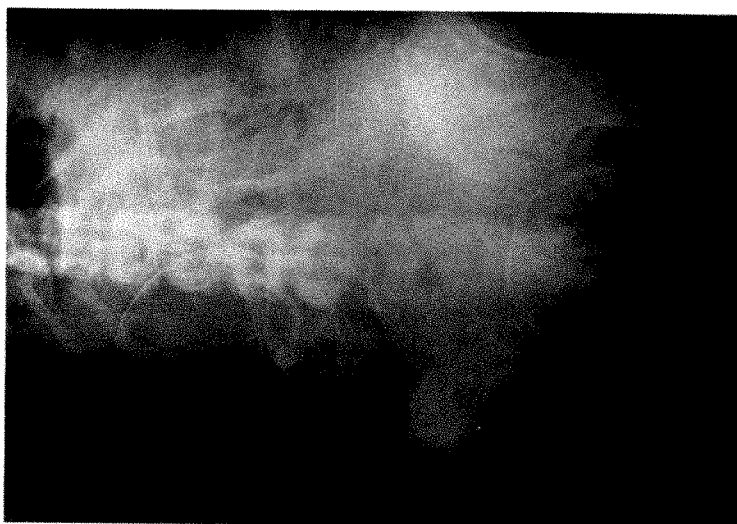


Fig. 4. Radiograph of twins of patient 1 in vertex/breech presentation before birth.

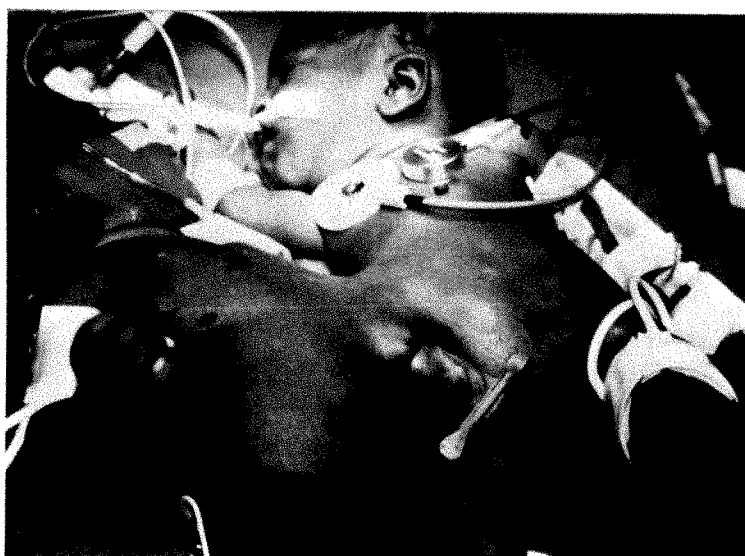


Fig. 5. Twins of patient 1 at birth.

second had scalp edema. To assist in antenatal evaluation, magnetic resonance imaging was performed at 20 weeks. A bulky low-lying anterior placenta and normal amount of amniotic fluid were detected. Because of the small fetal size and frequent fetal movements during the imaging process, specific fetal structures were not well identified. Concurrent sonographic evaluation revealed conjoined twins with separate heads and hearts and common thoracic cavity, abdominal structures, and pelvis. Two separate spines were noted. Twin B had marked hydrocephalus, a pericardial effusion, and ascites. Intrauterine fetal death occurred in the twenty-first week of pregnancy, and the patient was admitted with mild preeclampsia for delivery. Heavy vaginal bleeding developed during Pitocin ad-

ministration and, combined with a very unfavorable cervix, led to a decision to proceed with an abdominal delivery. Necropsy confirmed the presence of two heads, two upper chests, four arms and hands, one common abdomen, one umbilical cord, two right legs, one left leg, and one set of female genitalia, as previously discerned from sonographic evaluation.

Comment

Antenatal diagnosis of conjoined twins and appropriate planning are important factors in obtaining favorable outcomes for mother and infants. Herbert et al.¹ outlined a perinatal management protocol for conjoined twins, and we followed many of their principles in the management of the cases presented in this

report. We, however, added magnetic resonance imaging as an additional diagnostic modality to the use of sonography, amniocentesis, and amniography.

Magnetic resonance imaging employs the principles of nuclear magnetic resonance. Although first described in 1945, only recently have technologic advances allowed application of nuclear magnetic resonance to the imaging of complex living organisms. The word nuclear has been deleted, since there is no radiation exposure to the object being imaged. The preferred name of the technique is magnetic resonance imaging.

Many nuclei possess a magnetic moment and are in effect small magnets. In living systems, the hydrogen nucleus, occurring primarily in fat and water, is the most useful nucleus for imaging. Other biologically important nuclei are carbon 13, fluorine 19, sodium 23, phosphorus 31, and potassium 39. Their inherently lower nuclear magnetic resonance sensitivities and lower physiologic concentrations, however, make them less useful for magnetic resonance imaging studies.

In the absence of an externally applied magnetic field, the magnetic moments of the various atoms are randomly distributed. When a substance containing such nuclei is placed in a magnetic field, a resonance condition is produced in which the nuclei can absorb energy at a prescribed frequency from an electromagnetic wave called a radiofrequency pulse. When the incident radiofrequency pulse is switched off, the nuclei will reemit this energy at the same frequency, which can be measured with sensitive equipment. Because a given time is required for the nuclei to release the energy they have absorbed, one can obtain two sample-related time controls: the longitudinal (spin-lattice) relaxation time and the transverse (spin-spin) relaxation time. These relaxation times are affected by the chemical environment of the hydrogen nuclei. The magnetic resonance imaging gray scale is therefore as follows: air and cortical bone give the lowest intensity signal followed in increasing order by ligaments and tendons, muscle, brain/spinal cord, bone marrow, and fat as the brightest.¹¹ The reader is referred to review articles for additional background information on nuclear magnetic resonance.^{12, 13}

International experience with magnetic resonance imaging in human pregnancy is limited but expanding rapidly. Weinreb et al.⁷ presented five patients with abnormal pregnancies that demonstrated the complementary nature of magnetic resonance imaging to ultrasonography. In a gravid woman with oligohydramnios at 30 weeks' gestation, good fetal anatomic detail was noted in an infant with Potter's syndrome. Since magnetic resonance imaging does not depend on the amniotic fluid for successful visualization of the fetus, and since the fetus is not likely to move in the absence

of amniotic fluid, important information about associated anomalies may be provided. This is in contrast to sonography, which requires fluid-filled structures to provide an acoustic window. Fetal anomalies were also evaluated in a fetus with anencephaly and polyhydramnios and in a fetus with a large pelvic mass. Smith et al.⁵ used the technique in six first-trimester patients and proposed that magnetic resonance imaging may be relevant in the study of preeclampsia. Smith also stated that fetal tissue is displayed with greater detail than seen by ultrasound and, when coupled with the longitudinal relaxation time measurement, should provide a new method of fetal tissue analysis.

Johnson et al.⁶ noted that the attractiveness of magnetic resonance imaging to the obstetrician was its safety and its soft tissue definition. No significant hazard had been identified in the more than 2000 individuals undergoing imaging in the United Kingdom. In experiments exposing living cells to the conditions experienced during magnetic resonance imaging, no evidence was found of mutagenic or lethal effects in bacterial culture and there were no adverse chromosomal effects in human lymphocytes. They advised prudently to avoid imaging when rapid organogenesis was occurring. Furthermore, because each image took approximately 2 minutes, pictures of the fetus taken early in pregnancy were of poor quality because of fetal movement. In late pregnancy, when the fetus moves less, much better resolution of internal fetal structures is possible.

McCarthy et al.⁹ described magnetic resonance imaging and fetal anatomy and agreed that the best results were obtained in the third trimester because of decreased fetal motion, with the sagittal plane demonstrating fetal anatomy best. Transverse planes of fetal anatomy were easier to interpret when they were related to the global anatomy seen on sagittal images. Fetal fat, with its high-intensity signal, was easily seen and may provide another anthropometric evaluation of macrosomia or growth retardation. Placental size and location and umbilical cord insertion have been demonstrated, and the concentration of meconium has been measured *in vitro*.^{9, 14}

The two cases presented in this report demonstrate the comparative advantages of magnetic resonance imaging as a complementary diagnostic modality. Studies performed in the third trimester, when fetal movement has decreased and the fetus is larger, are superior to studies performed earlier in pregnancy. The magnetic resonance imaging of patient 1 predicted the skin-covered liver bridge found at separation of the twins. The large images and whole-body aspect of the images are especially invaluable in counseling the parents on the extent of abnormalities. The sagittal and coronal images are easily understood by nonradiologists and pa-

tients alike. In this respect the psychological advantage the magnetic resonance imaging gave the authors in preparing each set of parents for the respective outcomes cannot be underestimated.

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Maternal hemorrhage into the amniotic sac producing an apparent umbilical cord mass on sonogram

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A case of a mass around the fetus secondary to maternal hemorrhage and blood clot is presented. The sonographic features that allow distinction of a clot from a fetal teratoma are discussed. (*AM J OBSTET GYNECOL* 1986;155:649-51.)

Key words: Maternal hemorrhage, umbilical cord mass, ultrasound

We recently encountered a case in which a mass resembling a fetal teratoma appeared to arise from the fetal abdomen. Serial sonographic examinations and hematologic studies at the time of amniocentesis showed that the mass was a result of maternal bleeding into the amniotic fluid.

Case report

A 35-year-old white woman, para 1-0-0-1, experienced vaginal bleeding during the first and second trimesters. She was seen for genetic counseling because

of advanced maternal age at 17½ weeks' gestation. A 5.5 by 5.5 cm nonpulsatile complex mass was seen on a sonogram adjacent to and apparently arising from the ventral portion of the fetal abdomen. However, the mass moved separately from the fetus when the patient was shaken (Fig. 1) and the umbilical cord ran through its center, indicating it was not fetal in origin. At 18½ weeks' gestation, the mass had changed slightly in acoustical texture and size since the previous sonogram (Fig. 2).

At amniocentesis on the second visit, 20 ml of blackish amniotic fluid was withdrawn. The amniotic fluid was positive for hemoglobin A and negative for hemoglobin F. The amniocytes grew slowly but revealed a normal male karyotype.

A sonogram at 19½ weeks' gestation showed no mass but the amniotic fluid cavity was filled with mobile echogenic material. At 22½ weeks' gestation, there was clear amniotic fluid except for debris in the dependent por-

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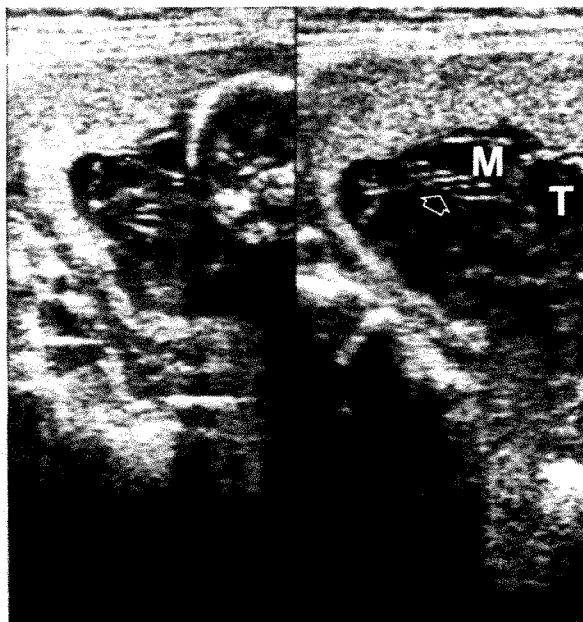


Fig. 1. Initial sonogram at 17½ weeks showing a 5.5 by 5.5 cm mass (M) adjacent to the ventral portion of the fetal abdomen with the cord running through the mass (arrow).

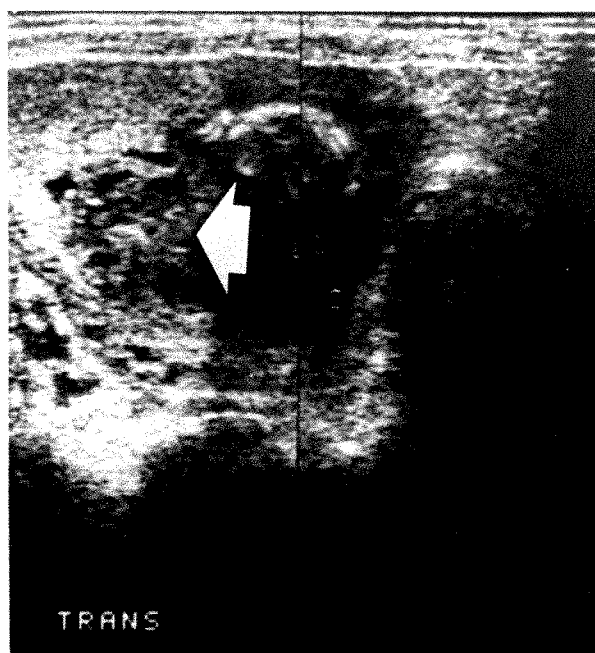


Fig. 2. Sonogram at 18½ weeks, showing the mass (arrow) to measure 7 by 4 by 4 cm and to have changed in acoustic texture since 17½ weeks.

tion of the amniotic cavity (Fig. 3). A sonogram performed at 30 weeks' gestation because the fundus appeared asymmetric to the right showed appropriate growth. The amniotic fluid volume had decreased. There was no evidence of uterine anomaly or extra-uterine pregnancy.

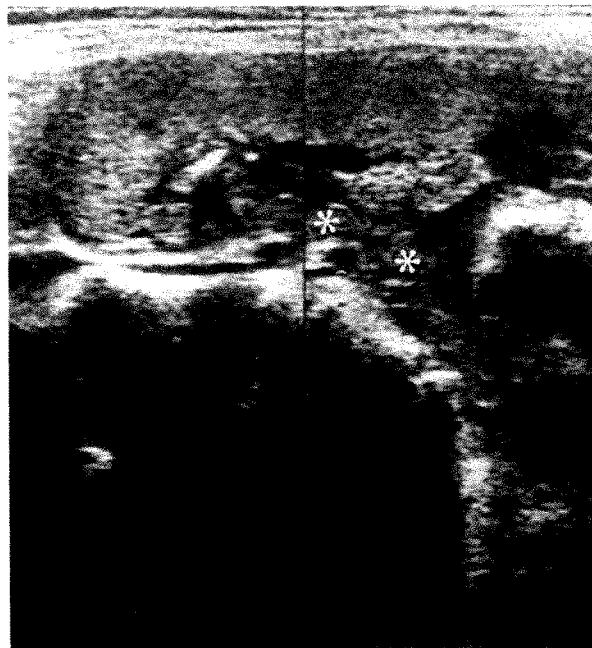


Fig. 3. Sonogram at 22½ weeks, showing debris (asterisks) in the dependent portion of the amniotic cavity.

At 31½ weeks' gestation, the fetus was delivered by cesarean section for chorioamnionitis. At the time of cesarean section, the uterus was asymmetric. The placenta and lower body of the fetus were within a sacculation located between the junction of the fallopian tube with the uterine wall and the uterine cavity with the chest and head of the fetus in the uterine cavity proper. Old clot was seen inside and outside of the membranes. A normal male infant weighing 1380 gm survived less than 24 hours before dying of hyaline membrane disease.

The mother was treated with ampicillin for chorioamnionitis and made an uneventful postpartum recovery.

Comment

The diagnosis of an intra-amniotic clot was made in utero (1) by observation of the alteration in the shape and pattern of the mass on serial sonograms and (2) by the ability to see the fetus move separately from the mass, thus distinguishing the mass from a fetal teratoma. It was shown to be of maternal origin by a maternal history of bleeding antedating the amniocentesis and a positive amniotic fluid test for hemoglobin A at the time of amniocentesis.

It is well known that blood clot changes rapidly in appearance when serial sonograms are performed. This feature distinguishes clot from neoplasm. The serial sonograms performed in our case showed the characteristic evolution of the hematoma from a dense group of echoes into a less echogenic lesion and finally the dispersal of the clot into the amniotic fluid where it was seen as multiple small mobile echoes.

The association of brown amniotic fluid at the time of second-trimester genetic amniocentesis with a history of vaginal bleeding is well known. Legge¹ reported that the pigments contributing to the discoloration include hemoglobin A, hemoglobin F, and myoglobin with only myoglobin being of poor prognostic significance.

It is surprising that purely maternal bleeding occurred into the amniotic sac. This phenomenon can occur if maternal blood dissects into the potential space between the chorion and decidua and then bursts through the amniotic membrane and enters the amniotic cavity. The site of entry may then seal over.

As far as we are aware, this is the most advanced example of an angular pregnancy examined sono-

graphically. It is disappointing that the appearances were identical to those of a normal intrauterine pregnancy. Although clinically the pregnancy was felt to be asymmetric in location, it appeared centrally placed on the sonogram. Interstitial pregnancy is acknowledged to be a difficult sonographic diagnosis.²

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Therapy of candidal vaginitis: The effect of eliminating intestinal *Candida*

Nystatin Multicenter Study Group

A total of 258 patients with candidal vulvovaginitis, all of whom also exhibited *Candida* organisms in the rectum, were treated for 1 week with vaginal tablets only (nystatin or clotrimazole) or with both vaginal and oral tablets (nystatin). Mycologic and symptomatic responses were superior for the group receiving combined intravaginal-oral therapy; the vaginas of 88% of those treated by both routes were cleared of *Candida*, as compared with 75% of those receiving only intravaginal medication ($p < 0.05$). Nystatin and clotrimazole were equally effective. When the 258 patients, regardless of treatment regimen, were grouped into those whose intestinal tracts after therapy contained *Candida* or those free of *Candida*, the response rates of the vaginal infection at all follow-up examinations favored the latter group ($p < 0.05$ to < 0.001). Vaginal infection recurred in 19.7% of patients treated only intravaginally and 14.7% of those receiving combined therapy. These results suggest the value of eliminating any intestinal reservoir of *Candida* when treating patients with candidal vulvovaginitis. (*AM J OBSTET GYNECOL* 1986;155:651-5.)

Key words: *Candida albicans*, intestinal candidiasis, vaginitis, nystatin, clotrimazole

Many factors, including use of oral contraceptives, pregnancy, and treatment with broad-spectrum antibiotics or corticosteroids, predispose the patient to vulvovaginal candidosis and complicate its management. It long has been suspected that the gastrointestinal tract also may play a role by affording a reservoir of *Candida* organisms capable of initiating or perpetuating this common and distressing infection.¹⁻⁴ However, the few studies conducted to date have failed to demonstrate

that the vaginitis is better managed by suppressing this intestinal reservoir with an antifungal agent.^{5,6}

The present multicenter study was undertaken to explore further the possibility that patients with candidal vaginitis will benefit if local (intravaginal) therapy is supplemented by removal of *C. albicans* from the lower intestinal tract. Ancillary to this primary objective, we sought to determine whether intravaginally administered clotrimazole has any significant advantage over the long-established use of nystatin, as has been reported by some⁷⁻¹⁰ but not by others.¹¹⁻¹³

Material and methods

Case material was provided by 10 gynecologists located in six countries. The clinical diagnosis of candidal vaginitis was confirmed in all 271 patients by a positive

Individual members of the Nystatin Multicenter Study Group are listed at the end of the article.

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Table I. Composition of study population

	Group 1— oral and intravaginal nystatin	Group 2— intravaginal nystatin	Group 3— intravaginal clotrimazole
No. of patients	83	86	89
Mean age (yr)*	30	31	30
Range	17-92	16-62	17-52
Married	63	63	62
Single	16	21	24
Divorced or widowed	4	2	3
Pregnant	11	11	15
Oral contraceptive use	23	25	23
No. of candidal vaginitis episodes in previous 12 months			
1	7	6	4
2-4	67	66	69
>4	4	8	6
Unknown	5	6	10

Table II. Response of candidal vaginitis to intravaginal therapy

	Visit 2		Visit 3		Visit 4	
	%	n	%	n	%	n
Clinical response*						
Clotrimazole (n = 89)	42	35/83	57	46/80	72	55/76
Nystatin (n = 86)	32	27/84	53	41/78	68	50/74
	NS		NS		NS	
Negative vaginal mycology						
Clotrimazole (n = 89)	61	51/83	74	59/80	75	57/76
Nystatin (n = 86)	69	58/84	76	59/78	76	56/74
	NS		NS		NS	

NS, Not statistically significant.

*Disappearance of signs and symptoms of infection.

potassium hydroxide examination of a vaginal swab specimen and also in all but 11 by a positive culture of *C. albicans*. Wet mounts and hanging drop preparations were obtained to exclude patients with vaginal infections caused by *Gardnerella vaginalis* and *Trichomonas vaginalis*, respectively. For the purpose of this study, material from a pretreatment rectal swab from each patient had to exhibit the pseudohyphae and budding yeasts of *Candida* on potassium hydroxide examination or produce a positive culture.

All patients qualifying for admittance to the trial received combined local (intravaginal) and oral treatment daily for 1 week as follows: Oral: group 1, nystatin; group 2, placebo; group 3, placebo. Local: group 1, nystatin; group 2, nystatin; group 3, clotrimazole.

Participating physicians assigned patients to group 1, 2, or 3 according to a random design. The oral medication (500,000 U nystatin tablets or matching placebo tablets) was given as two tablets three times daily, that is, 3,000,000 U daily. A single vaginal tablet containing either 100,000 U of nystatin or 100 mg of clotrimazole was inserted each night at bedtime. The 1-week course of treatment was not interrupted for menses.

Follow-up clinical and mycologic examinations (potassium hydroxide preparation and culture) to assess the response of the vaginitis were accomplished 1 to 7, 8 to 21, and 22 to 49 days after completion of therapy. At each of these time points (hereafter referred to as visits 2, 3, and 4, respectively) material from a rectal swab also was examined mycologically (potassium hydroxide preparation and culture).

Statistical analyses of the therapeutic responses observed with the three treatment regimens were performed with use of the Mantel-Haenszel approach to the analysis of rates and proportions.¹¹ Fisher's exact test was used to analyze all 2 × 2 tables.

Results

Of the 271 patients with proved vaginal candidiasis and coexisting intestinal *Candida*, 258 satisfactorily completed the study (83, group 1; 86, group 2; 89, group 3). Five patients in group 1 were lost to follow-up, as were one each from groups 2 and 3. Three patients each were excluded from groups 1 and 3 for violations of the study protocol. Most of the 258 patients had experienced at least two prior episodes of

Table III. Clinical and mycologic responses in candidal vaginitis

	Intravaginal therapy*		Intravaginal plus oral therapy†		Significance
	%	n	%	n	
Clinical response					
Visit 2	37	62/167	48	37/77	NS
Visit 3	55	87/158	80	61/76	p < 0.001
Visit 4	70	105/150	87	63/72	p < 0.01
Negative vaginal mycology					
Visit 2	65	109/167	79	61/77	p < 0.05
Visit 3	75	118/158	88	67/76	p < 0.05
Visit 4	75	113/150	86	62/72	NS

*Groups 2 and 3 combined.

†Group 1.

Table IV. Influence of continuing candidal colonization of intestinal tract* on response to therapy† for candidal vaginitis

	Visit 2		Visit 3		Visit 4	
	%	n	%	n	%	n
Clinical response						
Colonization	25	30/118	37	37/99	52	46/88
No colonization	55	69/126	82	111/135	91	122/134
	p < 0.001		p < 0.001		p < 0.001	
Negative vaginal mycology						
Colonization	52	61/118	69	68/99	65	57/88
No colonization	86	109/126	87	117/135	88	118/134
	p < 0.001		p < 0.05		p < 0.001	

*Determined at each visit by potassium hydroxide examination and culture of rectal swabbings.

†Clotrimazole vaginal tablets; nystatin vaginal tablets with or without nystatin oral tablets.

candidal vaginitis within the past year. Demographic and other characteristics of the three treatment groups generally were comparable (Table I).

The clinical and mycologic responses to therapy with only local nystatin (group 2) or clotrimazole (group 3) were similar at all three follow-up examinations (Table II*); therefore the findings for these two groups, that is, local therapy only, have been combined for comparison with group 1 (local therapy plus oral nystatin).

Table III compares the clinical and mycologic responses to intravaginal plus oral nystatin (group 1) and intravaginal therapy only (groups 2 and 3 combined). Although the differences were not statistically significant at every visit, group 1 consistently had a better response rate.

Rectal swab specimens obtained after therapy were negative for *Candida* in more than 70% of patients in group 1; the rectal contents also became free of *Candida* (presumably spontaneously) by the first follow-up examination in 42% of the combined group 2 and 3 pa-

tients. Therefore we divided the total study population into those whose intestinal tracts continued to show this organism and those cleared of it. As shown in Table IV, at all three follow-up examinations both the mycologic and clinical response rates of the vaginitis were significantly higher when the intestinal tract was free of *Candida*.

Mycologically confirmed recurrence of candidal vulvovaginitis occurred by the second or third follow-up visit in 11%, 22%, and 16% of group 1, 2, and 3 patients, respectively, who were originally "cured" (that is, had a negative vaginal mycologic examination at the first follow-up visit). Recurrences were observed in 14.7% (16/109) of all such patients whose rectal potassium hydroxide preparations and cultures at the first follow-up were negative and in 19.7% (12/61) of those whose rectums contained *Candida* ($p > 0.05$).

All three drug regimens were well tolerated.

Comment

In the present study monotherapy with nystatin or clotrimazole vaginal tablets produced similar responses in patients with candidal vulvovaginitis. This was so despite the fact that nystatin therapy was continued for

*The numbers of patients at visits 2, 3, and 4, as shown in Tables II to IV, differ somewhat because not all patients presented for all follow-up visits.

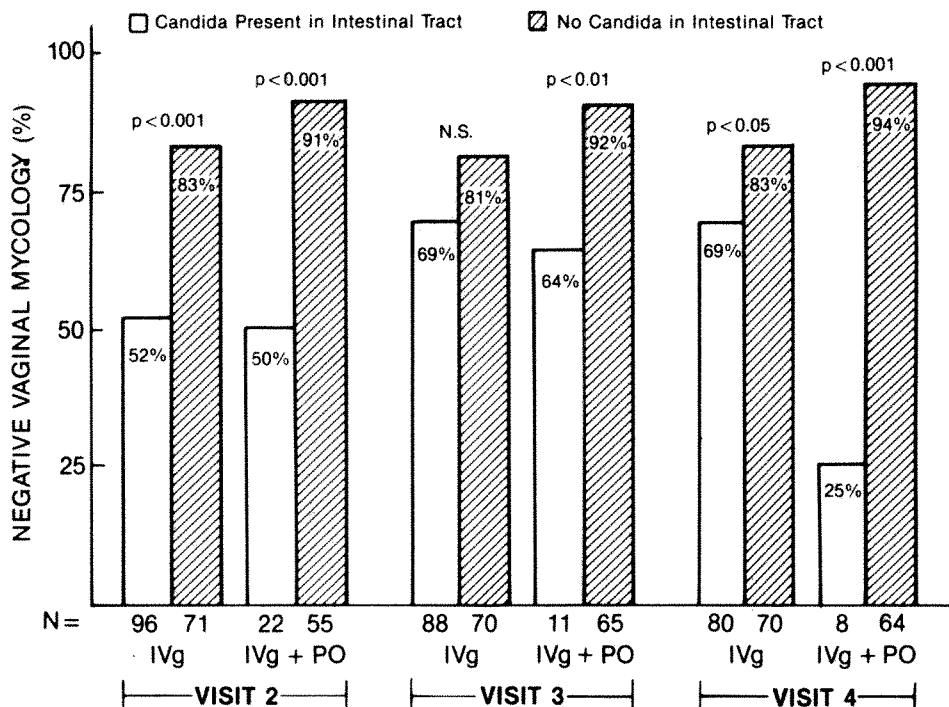


Fig. 1. Response to therapy of candidal vaginitis as related to presence of *C. albicans* in the intestinal tract. IVg = Intravaginal therapy only; IVg + PO = intravaginal plus oral therapy; open bar = *Candida* present in intestinal tract; hatched bar = no *Candida* in intestinal tract.

only 1 week, a short course that in previous studies provided responses inferior to those obtained with clotrimazole.⁷⁻¹⁰ Because of the similarity of the findings with both drugs the two treatment groups could be merged for comparison with the treatment regimen combining intravaginally and orally administered nystatin. Predictably the latter regimen cleared the intestinal tract of the *Candida* organism in almost 70% of patients (negative rectal culture and potassium hydroxide preparation at both first and second follow-up visits.) Close to 35% of the patients receiving only intravaginal therapy also exhibited *Candida*-free rectal specimens at both follow-up visits, an unexpected finding also reported earlier by Lebherz et al.¹⁵ among patients receiving local therapy for candidal vulvovaginitis.

The response rate of the patient population that received the combined local-oral therapy consistently was superior to that of those receiving only local therapy. Among the patients in both treatment groups the clinical and mycologic response rates were similarly and significantly improved when the intestinal tract was free of *Candida* (Fig. 1). In fact, the odds¹⁶ of obtaining a mycologically confirmed cure favored patients whose rectums had been cleared of this organism by at least two to one. The fact that this situation prevailed twice as frequently in the combined local-oral treatment group accounts for the better results obtained with the

latter therapy. The better response rate in patients whose intestines became *Candida*-free is consistent with the earlier observation of Lambotte et al.¹⁷ that patients with candidal vaginitis responded better to local therapy if the organism was not present in their rectums before therapy.

It also was of interest to compare the rapidity of response of the vaginitis to therapy and the frequency of recurrence in patients whose intestinal tracts did or did not afford a reservoir for the *Candida* organism. To this end we reviewed the findings at the first follow-up visit (1 to 7 days after completion of therapy) for all patients who ultimately responded to therapy, that is, vagina was negative for *Candida* at the second follow-up (8 to 21 days after therapy). Clinical and/or mycologic responses were observed in 74% of the 88 patients exhibiting *Candida* in rectal specimens at either the second or third follow-up examination and in 94% of the 85 patients with negative rectal specimens at both visits ($p < 0.01$). Thus it appears that not only is the response of the vaginitis to therapy more consistent, but also it occurs more rapidly when there is no so-called intestinal reservoir of *Candida*.

Recurrences of the vaginitis were observed in only 28 of the 170 patients whose vaginas were negative for *Candida* at the end of therapy. The brevity of the follow-up period (up to 49 days) may account for this low recurrence rate, and in turn the latter may account

for the lack of difference in the frequency of recurrences in patients whose rectums were colonized with *Candida* and those who were free of this organism.

The present study, in which significantly better therapeutic responses were obtained in vulvovaginal candidiasis when the intestinal tract was rid of *C. albicans*, gives clinical significance to earlier observations of a positive association between the presence of *Candida* in the vagina and in the rectum.^{1, 2, 4, 18}

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Treatment of cornual pregnancy with methotrexate: Case report

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Presented is the case of a woman with a cornual pregnancy documented by ultrasound and laparoscopy and successfully treated with an 8-day course of methotrexate. (AM J OBSTET GYNECOL 1986;155:655-7.)

Key words: Ectopic pregnancy, methotrexate

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Successful treatment of ectopic pregnancy by chemotherapeutic means has been infrequently recounted in the literature. However, several authors have suggested its usefulness in appropriate cases where surgical management might impair future infertility^{1, 2} or be of significantly greater risk to the patient.² Presented is a

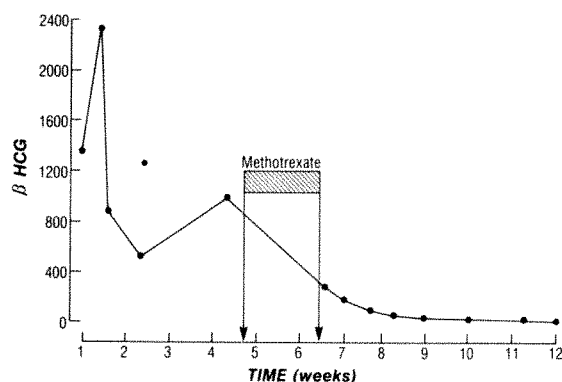


Fig. 1. β -hCG levels in milli-international units over time.

case of cornual pregnancy treated with methotrexate after initial erroneous diagnosis as gestational trophoblastic disease.

Case report

J. H. was a 33-year-old married white woman, gravida 1, para 1, who was seen in June 1985 complaining of vaginal spotting since a normal menstrual period on June 11, 1985. A urinary monoclonal pregnancy test was positive. Examination revealed a normal uterus and questionable enlargement of the right adnexa. A thorough curettage identified neither chorionic villi nor fetal parts. Pelvic ultrasound examination was reported as normal. A quantitative serum determination of the β -subunit of human chorionic gonadotropin (β -hCG) was 1350 mIU on June 25, 1986. Diagnostic laparoscopy was performed and showed no specific evidence of ectopic gestation except a slight asymmetry of the right cornua. A free flow of indigo carmine was noted from both distal tubes. β -hCG on July 1 and July 3 were 800 and 507 mIU, respectively. However, on July 18 the value had risen to 988 mIU (Fig. 1). Because of the presumed diagnosis of gestational trophoblastic disease, she underwent a standard 8-day course of methotrexate with citrovorum factor rescue at the New England Center for Trophoblastic Disease. Repeat ultrasound examination during her treatment was more suggestive of a persistent right cornual pregnancy. This diagnosis was confirmed with diagnostic laparoscopy. Because there was no evidence of imminent rupture and β -hCG levels were falling, the course of methotrexate was completed.

Follow-up β -hCG levels during the next several weeks returned to zero. A follow-up hysterosalpingogram showed a filling defect in the area of the right cornu with a communication originating in the interstitial portion of the tube and extending laterally and superiorly to form a blind, irregularly contoured sac. The laparoscopy confirming cornual pregnancy demonstrated an area of symmetrical enlargement of the right uterine cornual area consistent in location and size with the filling defect noted on the hysteroqram (Fig. 2).



Fig. 2. Hysterosalpingogram showing filling defect at the right cornu.

Comment

This case supports the earlier contention that methotrexate can be used successfully and with minimal side effects in the treatment of ectopic gestation.^{1,2} In many cases, surgical treatment may be either contra-indicated for medical reasons or unattractive in light of desire for future fertility. An alternative to surgical resection would be very useful.

The effects of methotrexate on future fertility and pregnancies were discussed by Walden and Bagshawe³ in 1976 and Shinkai et al.⁴ in 1981. Walden and Bagshawe noted a higher incidence of anomalies in future pregnancies, although no increased difficulty with conception. Shinkai et al. showed no incidence of anomalies in their series of 45 patients. On the other hand, the problems of impaired fertility associated with surgical resection, particularly with cornual pregnancies, were recognized by Schenker et al.⁵ in 1972. Finally, the well-known side effects of methotrexate, which include bone marrow depression and stomatitis, need to be weighed against benefits gained by nonsurgical treatment. Dosage and number of courses of treatment are a signifi-

cant factor. In this case, one 8-day course without any significant ill effects was sufficient. For now, evidence supports the usefulness of methotrexate for unruptured ectopic pregnancies in anatomic locations requiring more extensive surgical resection where fertility is desired and/or in circumstances where operation might be contraindicated.

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Severe peripheral arteriospasm following oxytocin administration

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A parturient patient with imminent gangrene of the extremity caused by oxytocin-induced arteriospasm is described. The appliance of conventional measures such as anticoagulation and vasodilatation produced an immediate and sustained reversal of the arteriospasm together with dramatic relief of symptoms and signs. (*AM J OBSTET GYNECOL* 1986;155:657-8.)

Key words: Arteriospasm, oxytocin, side effect

The amounts of oxytocin administered for most obstetric purposes are insufficient to produce marked alterations of blood pressure. When large amounts are administered for elective abortion or during a uterine surgical procedure, however, a marked fall in arterial blood pressure may occur because of a direct relaxing effect on vascular smooth muscle. Severe local angiospasm as described in the present case has not been reported previously.

Case report

A 33-year-old woman, gravida 6, para 3, abort uses 2, with one living child was scheduled for elective cesarean section because of prior Shirodkar procedure for cervical incompetence. The past history was unre-

markable, with no drug ingestion or sensitivity. Obstetric history revealed six pregnancies with only one normal term delivery; four pregnancies terminated in either spontaneous abortion at weeks 9 and 13 or early delivery at 24 weeks' gestation. The oxytocin administration in four previous pregnancies was without incident. Three months following the fifth pregnancy the diagnosis of cervical incompetence was confirmed by hysterosalpingography. The course of the present (sixth) pregnancy was uneventful and at 11 weeks of gestation the Shirodkar procedure of cervical cerclage was performed with use of general anesthesia (4 mg/kg of sodium thiopental maintained with a 50% mixture of nitrous oxide and oxygen). At 39 weeks of gestation the patient was admitted for elective cesarean section. Physical examination at this time was normal: blood pressure, 105/70 mm Hg; pulse, 100 and regular; temperature, 36.7° C; hemoglobin, 14 gm/dl; hematocrit, 42.7%; white blood cell count, 16,250 mm³; thrombocytes, 250,000 mm³; prothrombin time, 87%; partial thromboplastin time, 40 seconds; serum electrolytes liver and kidney function, within normal ranges; urinalysis results, normal. Lower uterine segment cesarean section was performed with 20 ml of

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bupivacaine epidural anesthesia administered via a Tuohy needle at the L1-2 interspace. A normal term 3140 gm infant, with Apgar scores of 10 and 10 at 1 and 5 minutes, was delivered. Oxytocin infusion was begun following delivery at a dose of 10 mU/min via intravenous catheter inserted to a superficial vein on the dorsum of the left hand. Fifteen minutes later a sustained progressive arteriospasm of the left hand and later of the distal two thirds of the left forearm also appeared as a cyanotic cold pulseless extremity, resulting in blackening of the fingers 15 minutes later. Normal brachial and absent radial artery pulsation was noted. The oxytocin infusion was discontinued, and the intravenous catheter was withdrawn. Brachial and radial intra-arterial injections of 3 ml of 1% lidocaine and 2 ml of 2% papaverin were given. In addition, an intravenous injection of 3 ml of 1% lidocaine, 2 ml of 2% papaverine, and 10,000 IU of heparin was provided. Three minutes following the intra-arterial injection a sustained progressive improvement was achieved in color, temperature, and pulse of the distal third of the left arm and hand. Severe edema of the arm and hand, developing within 24 hours, disappeared gradually in 5 days. During that period the left arm was elevated and a magnesium sulfate compress applied. The post-cesarean course was uneventful, and vasospasm did not recur. The patient was discharged from hospital at the eighth day of hospitalization without any residual damage, except for some puffiness and sensitivity of the hand.

Comment

Oxytocin is a synthetic polypeptide hormone containing eight amino acids with a molecular weight of about 1000. It is normally produced in the hypothalamus. The oxytocic drugs such as Syntocinon and Pitocin are currently prepared by synthesis. Their effects are executed mainly on uterine smooth muscle. They are devoid of posterior pituitary vasopressin effect. Large amounts may cause a decrease in systolic and especially diastolic blood pressure, flushing, and an increase in limb blood flow.¹ When large doses are ad-

ministered for elective abortion or during a uterine surgical procedure, a marked fall in arterial pressure may occur, particularly in deeply anesthetized patients.

The vasodilator effect of oxytocin is independent of autonomic receptors. Infusion of oxytocin within a physiologic dose was found to cause a marked increase in renal blood flow in dogs.² Another effect that was found with large doses is an antidiuresis resulting from the slight antidiuretic hormone-like activity of the hormone. When excessive intravenous fluids are administered (mainly 5% glucose), water intoxication may occur.

The above case is unique in that a severe local arteriospasm accompanied the routine minimal dose of oxytocin infusion required for uterine contraction following delivery. To the best of our knowledge such complication has not been reported in the English literature. The oxytocin used today is a synthetic hormone with a very specific action on the uterine smooth muscle. Smooth muscle of tissues other than uterus blood vessels and mammary myoepithelium is not sensitive to oxytocin. However, in experimental studies oxytocin-like vasopressin in high doses has effects on numerous tissues.³

Oxytocin sensitivity may have caused this reaction in our case. Treatment of severe arteriospasm includes anticoagulants, vasodilators, and sympatholytic agents, in addition to withdrawal of the offending agent.

More resistant cases, with increasing cyanosis, may require such procedures as epidural and stellate ganglion blocks and mechanical intra-arterial dilatation as reported for ergotamine-induced arteriospasm.

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Uterine arteriovenous malformation successfully embolized with a liquid polymer, isobutyl 2-cyanoacrylate

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A uterine arteriovenous malformation was diagnosed angiographically in a 27-year-old woman presenting with recurrent menometrorrhagia. Bilateral hypogastric artery embolization with a liquid polymer, isobutyl 2-cyanoacrylate (Bucrylate), resulted in subsequent normal menses. Bucrylate offers a number of advantages over other agents previously used to embolize uterine arteriovenous malformations. (AM J OBSTET GYNECOL 1986;155:659-60.)

Key words: Uterine arteriovenous malformation, hypogastric embolization, Bucrylate, isobutyl 2-cyanoacrylate, pelvic angiography

Uterine arteriovenous malformations are uncommon but potentially life-threatening lesions. In the past, most have been managed surgically—by hysterectomy with or without hypogastric artery ligation. It has been reported recently that successful pregnancy could be accomplished after nonsurgical embolization of a choriocarcinoma-related pelvic arteriovenous fistula.¹ We report a uterine arteriovenous malformation successfully embolized with a liquid agent, isobutyl 2-cyanoacrylate (Bucrylate, Ethicon, Inc., Sommerville, New Jersey).

Case report

The patient was a 27-year-old woman, para 0-1-3-1, transferred to our medical center with the diagnosis of uterine arteriovenous malformation. Curettage had been performed at another institution because of an intractable vaginal hemorrhage. During the curettage, a hysteroscope was introduced and resulted in massive bleeding. Intramuscular prostaglandins were administered and emergency pelvic angiography performed. This revealed a large uterine arteriovenous malformation with blood supply from both hypogastric arteries. Blood was transfused and the patient transferred for embolization with a liquid agent, isobutyl 2-cyanoacrylate.

Past history was significant in that the patient had had multiple curettages as an adolescent because of heavy vaginal bleeding; multiple units of blood had been transfused. Voluntary first-trimester abortions were performed at ages 15 and 16. At age 21, the pa-

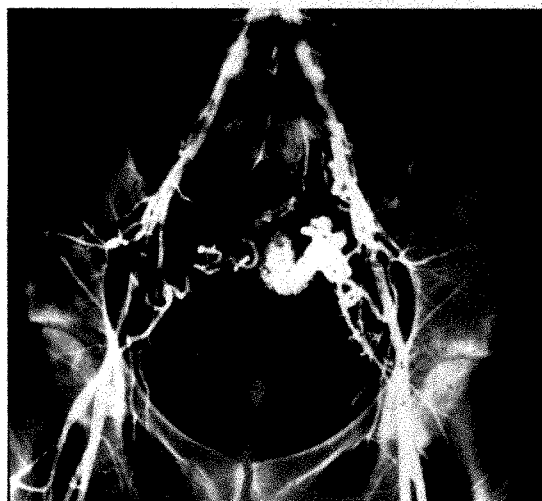


Fig. 1. Preembolization pelvic arteriogram demonstrating a large uterine arteriovenous malformation receiving most of its blood supply from the left hypogastric artery.

tient was delivered vaginally of a viable fetus at 30 weeks' gestation. Two years later, a spontaneous abortion occurred at 8 weeks' gestation.

Many attempts were made to control the patient's recurrent menometrorrhagia with various estrogen-progestin combinations. This most recent admission for hemorrhage occurred while the patient was using oral contraceptives. There was no history of intrauterine contraceptive device use.

Physical examination was within normal limits. The uterus was anteverted, normal in size, and nonpulsatile. The adnexa were negative to palpation. A moderate amount of vaginal bleeding was noted.

Hysterectomy was discussed as definitive therapy but the patient expressed a desire to retain the uterus if at all possible to attempt future pregnancy. After informed consent was obtained, the patient was taken to

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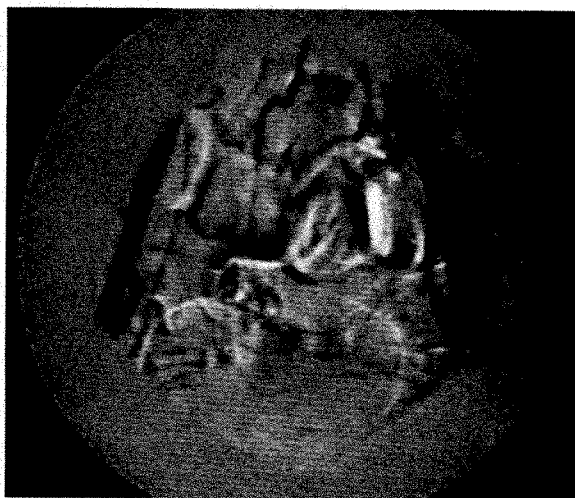


Fig. 2. Subsequent digital flush angiogram of the pelvis demonstrating no filling of the arteriovenous malformation and proper placement of the bilateral isobutyl 2-cyanoacrylate casts.

the angiography suite where, through a left axillary approach, both hypogastric arteries were selectively catheterized. Angiography confirmed a large uterine arteriovenous malformation receiving most of its blood supply from the left hypogastric artery (see Fig. 1). Both hypogastric arteries were embolized with isobutyl 2-cyanoacrylate. Postembolization arteriography confirmed proper placement of the Bucrylate casts (see Fig. 2). Her course was complicated by transient paresthesia and paralysis of the left arm, thought to be due to either a stretch injury of the brachial plexus or a hematoma in the plexus sheath. All symptoms resolved during the next few days.

Since embolization, the patient has had normal menses without excessive bleeding and no pelvic complaints. She receives no medication and has not attempted pregnancy. The patient has chosen barrier contraception.

Comment

Uterine arteriovenous malformation may be congenital or acquired. Acquired malformations may be due to previous uterine trauma (prior pelvic operation, curettages), intrauterine contraceptive devices, pathologic pregnancy-related events, and the treatment of gestational trophoblastic disease. This patient's history of menometrorrhagia as an adolescent requiring blood transfusions and multiple curettages may indicate a congenital etiology although trauma related to those curettages may also play a role.

There are two case reports in the literature of pregnancy after embolization of a uterine arteriovenous malformation. Forssman et al.² at laparotomy ligated all branches of the left internal iliac artery except the uterine, through which they embolized Gelfoam; the patient had had a previous laparotomy with hysterotomy and suturing of a bleeding artery. She was delivered electively by cesarean section at 38 weeks of a viable 3100 gm infant. No abnormalities were noted at the time of delivery. Chapman and Lutz¹ nonsurgically embolized a large arteriovenous malformation of the uterus that occurred after successful treatment of a choriocarcinoma with chemotherapy. They used a combination of Gelfoam and Gianturco coils to embolize both hypogastric arteries. The patient subsequently was delivered by cesarean section at 32 weeks' gestation of a 1240 gm infant. Pregnancy was complicated by placenta previa with premature labor and hemorrhage. At operation, there was no evidence of any abnormalities except for the presence of abnormal placentation (placenta previa) with antepartum hemorrhage.

Isobutyl 2-cyanoacrylate (Bucrylate) is a tissue adhesive that polymerizes instantaneously when in contact with ionic solutions and produces permanent vessel occlusion. It offers a number of theoretical advantages over mechanical embolization devices (Gianturco coils) and particulate embolizers (Gelfoam). Because it is a liquid, it can be delivered via microcatheters and can be used for superselective permanent vascular occlusion. It can be used in both slow-flow and high-flow vascular lesions to effectively occlude the nidus of the lesion rather than just feeding vessels, as is accomplished with proximal embolization or surgical ligation. Permanent obliteration of the nidus should markedly reduce the likelihood of recurrence. Its rapid polymerization makes it effective independent of the patient's own clotting mechanisms, the latter being important in the mechanism of action of coils.

Isobutyl 2-cyanoacrylate is currently an investigational drug awaiting Food and Drug Administration approval for clinical use.

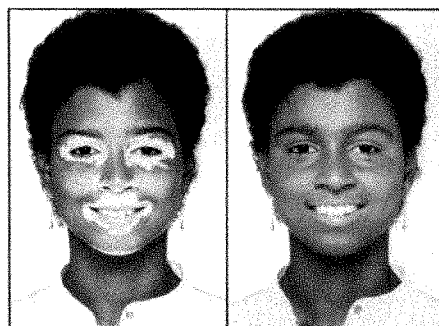
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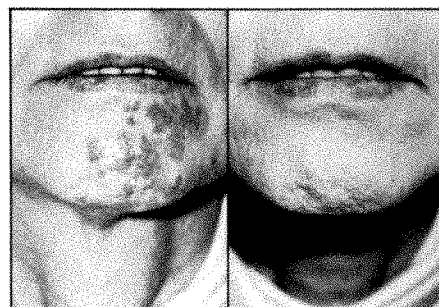
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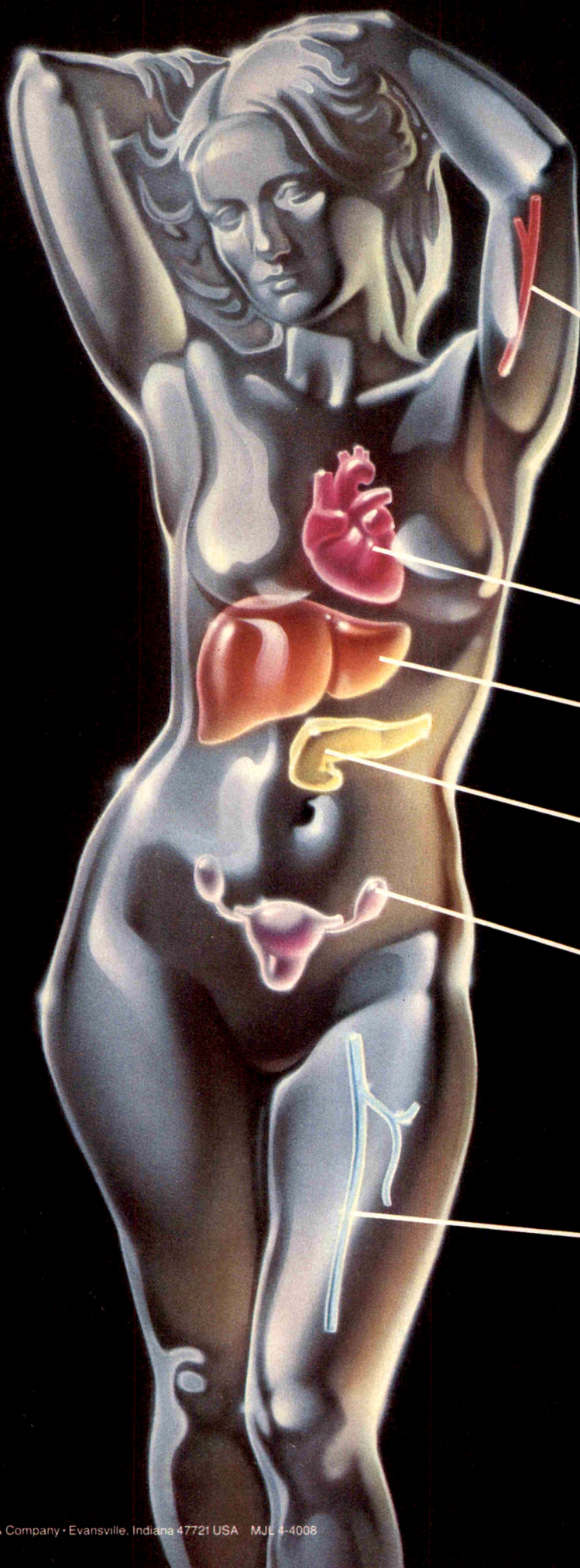
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†As noted in the labeling required of all oral contraceptives, an increase in blood pressure has been reported in patients receiving oral contraceptives. Increased blood pressure may lead to hypertension, defined as sustained pressure greater than 140/90 mm Hg. These data are from the case reports of 13 investigators and were analyzed by Mead Johnson personnel.

‡As noted in the labeling required of all oral contraceptives, certain liver function tests may be affected by estrogen-containing oral contraceptives, including increased sulfobromophthalein retention and increased prothrombin. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives; a few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

§As noted in the labeling required of all contraceptives, a decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The findings in these studies represent preliminary information and are yet to be confirmed. Prolonged follow-up will be necessary to see if clinical differences in processes such as atherogenesis can be observed in patients receiving OVCON-35.

¶It is generally accepted that all combination oral contraceptives act primarily by suppressing the pituitary-ovarian axis. The significance of different degrees of such suppression for the overall safety of any oral contraceptive steroid is not known. The oral contraceptive product prescribed for any patient should be one with the lowest estrogen content compatible with an acceptable pregnancy rate and patient acceptance.

#As noted in the OVCON labeling, a tendency toward increased blood coagulability has been observed in a significant percentage of patients on oral contraceptives. The findings in this present study represent preliminary information and are yet to be confirmed. Prolonged follow-up will be necessary to see if clinical differences in coagulation states can be observed in patients receiving OVCON-35.

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Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/tocemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk of fatal infarction compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg, or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000, ages 35-44, 33/100,000, ages 45-49, 140/100,000). The risk being concentrated in older women, in the use with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/tocemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision, sudden onset of proptosis or diplopia, papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and

there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gall Bladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) **Ectopic Pregnancy.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breast Feeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthaltein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T₄ by column, or T₄ by radioimmunoassay. Free T₃ resin uptake is decreased, reflecting the elevated TBG. Free T₄ concentration is unaltered. d. Decreased pregnenolone excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD-C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD-C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**). Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis; and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Mature solid teratoma associated with gliomatosis peritonei

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Presented is a 10-year follow-up of a case of gliomatosis peritonei (grade 0) complicating a mature solid teratoma (grade 0). (AM J OBSTET GYNECOL 1986; 155:661-2.)

Key words: Gliomatosis peritonei, mature solid teratoma

Gliomatosis peritonei is a miliary implantation of mature glial tissue on the peritoneum or omentum. Grossly, it is characterized by multiple small, gray-white, superficial nodules usually focally involving the pelvic peritoneum and adjacent omentum. Microscopically the implant consists of multiple nodules of mature glial tissue. The glial nodules may arise secondary to capsular defects with subsequent implantation or via lymphatic spread. The glial implants usually persist unchanged but may undergo fibrosis and disappear. When associated with immature teratoma, teratomatous and immature glial tissue has been found at subsequent biopsy. It is unknown if this is a direct transformation or secondary to inadequate initial sampling.

Case report

M. C., a 12-year-old girl, gravida 0, 6 months post menarche, presented with a nontender abdominal mass

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extending 3 cm above the umbilicus. Metastatic radiologic survey was negative. At exploratory laparotomy a 2080 gm, 20 by 15 by 19 cm left ovarian tumor was excised. Biopsies of multiple small gray implants studying the pelvic peritoneum were performed. Histologic examination of the ovarian tissue revealed mature epithelial, mesodermal, and endodermal elements with the vast majority of the tumor consisting of mature glial tissue. No mitoses were seen within the glial tissue (grade 0). The peritoneal implants were composed of nodules of mature glial tissue (grade 0) (Fig. 1). At 6- and 18-month follow-up, diagnostic laparoscopy with multiple biopsies revealed the continued presence of mature glial nodules in the pelvic peritoneum. There is no evidence of disease at 10-year follow-up.

Comment

Three previously reported cases are summarized in Table I. All four patients have survived with minimum follow-up of 4 years. Although gliomatosis peritonei complicating mature solid teratoma appears to be a benign condition, sampling to exclude teratomatous or immature glial implants is mandatory and follow-up laparoscopy with biopsy is recommended. Gliomatosis peritonei may also be associated with immature tera-

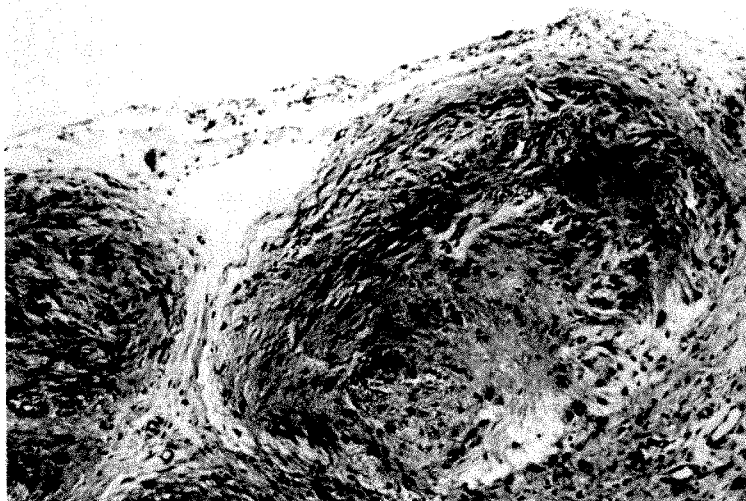


Fig. 1. Peritoneal implants; nodules of mature glial tissue.

Table I. Cases of mature solid teratoma with gliomatosis peritonei

Series	Age (yr)	Treatment	Follow-up (yr)
Shafie (J Surg Oncol 1984;27:18)	12	USO and omentectomy	5
Robboy (Hum Pathol 1970;1:643)	11	TAH, BSO, chemotherapy	4
Thurlbeck (Cancer 1960;13:804)	18	TAH, BSO, RT	37
Present case	12	USO	10

USO = unilateral salpingo-oophorectomy; TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy; RT = radiotherapy.

toma. The literature review of Truong et al.¹ reveals that gliomatosis peritonei tends to impart a favorable prognosis when it complicates immature teratoma (three deaths in 30 cases).

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Midtrimester diagnosis of endocardial fibroelastosis and atrial septal defect: A case report

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We present a case report of endocardial fibroelastosis combined with atrial septal defect in which the diagnosis was strongly suspected at 25 weeks of gestation. To our knowledge the only previous report of prenatal diagnosis of endocardial fibroelastosis was of one made at 36 weeks of gestation. (*AM J OBSTET GYNECOL* 1986;155:662-3.)

Key words: Echocardiography, endocardial fibroelastosis

Over the past few years, echocardiography has been used in the detection of cardiac malformations in utero. A variety of cardiac anomalies have been described.¹ In this case report the diagnosis of endocardial fibroelastosis combined with atrial septal defect was made at 25 weeks of gestation. To our knowledge the only previous report of prenatal diagnosis of endocardial fibroelastosis was of one made at 36 weeks' gestation.²

Case report

A 23-year-old woman, gravida 2, para 1, was referred to our hospital at 25 weeks' gestation because of suspected polyhydramnios. Real-time sector scanning

(Diasonic DRF-400) revealed polyhydramnios. The biparietal diameter and femur length of the fetus corresponded to 25 weeks' gestation. Cardiomegaly and significant ascites were detected. Real-time directed M-mode echocardiography revealed severe cardiac enlargement, especially of the left ventricle, which was globular in shape; the systolic and end-diastolic diameters were approximately twice the normal diameter, according to the gestational age. (Left ventricle: systolic diameter = 14 mm, end-diastolic diameter = 18.4 mm; right ventricle: systolic diameter = 7.4 mm, end-diastolic diameter = 10.4 mm.) The atrioventricular valves and the interventricular septum were thickened. The endocardial surface of the left ventricle was very echogenic (Fig. 1). The atria were moderately enlarged. Large atrial septal defect was noted. The ventricular outlets were normal with a good functioning of the aortic and pulmonaric valves. Fetal cardiac rhythm was 150 to 160 bpm. No other fetal malformations were detected.

These echocardiographic findings were indicative of

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endocardial fibroelastosis and atrial septic defect. The differential diagnosis included intrauterine myocarditis. Coarctation of aorta and valvular anomalies were ruled out.

We suggested termination of the pregnancy, but the parents objected. To improve cardiac output, digitalization was performed by oral digoxin.

At 31 weeks' gestation, in spite of a high-maintenance dose of digoxin (0.5 mg/day) for 6 weeks, there was no improvement in the fetal heart size and the ascites. (Left ventricular diameter continued to be approximately twice the normal size for the gestational age.) Fetal growth was in the normal range, and digoxin administration was stopped. Three weeks later the woman was admitted because she complained of reduced fetal movements. Ultrasonography revealed fetal death. Induction of labor was done with use of oxytocin infusion, and a hydropic dead male fetus, weighing 2400 gm, was delivered.

Pathologic examination confirmed the antenatal diagnosis of endocardial fibroelastosis and large ostium secundum-type atrial septal defect.

Comment

Endocardial fibroelastosis is a rare congenital heart disease and a well-known cause of congestive heart failure in early infancy. It is classified into two general types: primary and secondary. In primary endocardial fibroelastosis there is no valvular disease or other congenital cardiac abnormality. In the secondary type, left-sided obstruction congenital heart disease is noted.

The incidence is approximately 1% of congenital heart disease. Pathologically there is a white, opaque fibroelastic thickening of the endocardium and the valves. Microscopically a hyperplasia of the fibrous and elastic tissue of the endocardium is seen. The etiology is unknown, but several concepts of pathogenesis have been suggested: (1) viral infection, (2) congenital development defect, (3) anoxia, (4) deposition of fibrin, and (5) genetic factors.

The clinical manifestations chiefly include the signs

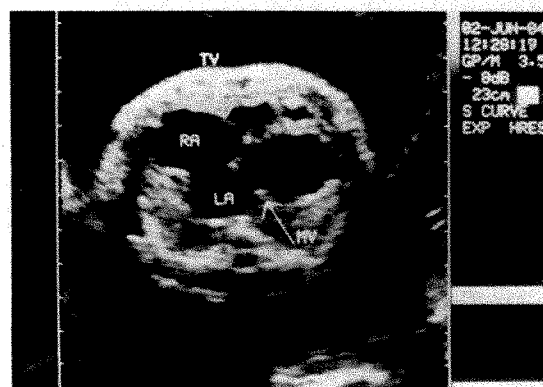


Fig. 1. Four-chamber view demonstrates the enlarged globular left ventricle and the atrial septal defect. RA = Right atrium. LA = Left atrium. MV = Mitral valve. TV = Tricuspid valve.

and symptoms of various stages of heart failure. Treatment includes use of digitalis and diuretics. The prognosis is poor, and most fetuses eventually succumb. Suggested echocardiographic findings for the diagnosis of primary endocardial fibroelastosis include: (1) ventricular diameters twice as large as in controls (especially left ventricle); (2) signs of congestive heart failure, such as ascites and pericardial and pleural effusion; (3) strongly echogenic endocardial surface; (4) lack of congenital heart disease of the left obstructed type, for example, aortic stenosis and coarctation of aorta.

The differential diagnoses include myocarditis, glycogen storage diseases of the heart, hypertrophic cardiomyopathy, and congestive cardiomyopathy. Whenever an early prenatal diagnosis of endocardial fibroelastosis is made, we suggest termination of the pregnancy.

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Midtrimester amniotic fluid delta optical density at 450 nm in normal pregnancies

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Spectrophotometric analysis of amniotic fluid has become the standard for assessing the fetal condition in Rh-immunized pregnancies. Serial amniocentesis is usually started at 28 to 29 weeks of gestation unless the antibody titer or history indicates it should be done earlier. This study presents the values from 14 to 20 weeks in normal pregnancies, which will also assist in evaluating pathologically elevated values. (AM J OBSTET GYNECOL 1986;155:664-6.)

Key words: Delta optical density at 450 nm, amniotic fluid bilirubin

Spectrophotometric analysis of amniotic fluid is a well-established laboratory test for the diagnosis and management of Rh-sensitized pregnancies. Since the classic work of Liley^{1,2}, this test has become the standard for assessing fetal condition in Rh-immunized pregnancies. Serial amniocenteses are usually begun at 28 to 29 weeks' gestation, unless the antibody titer or history indicates that the procedure should be done earlier.³

The three-zone graph of Liley for predicting the severity of disease included values only as early as 27 weeks' gestation. Today, with sonographic guidance, intrauterine transfusions can be done considerably earlier in severely immunized pregnancies. But little information is available concerning normal values of delta optical density at 450 nm (ΔOD_{450}) in early pregnancy. This study presents the values from 14 to 20 weeks' gestation in normal pregnancies.

Material and methods

Two hundred four amniotic fluid samples were obtained from normal pregnant women undergoing amniocentesis for advanced maternal age (35 to 39 years). All of the procedures were done by two investigators (S. L. W. and J. T. Q.). Only those women who were

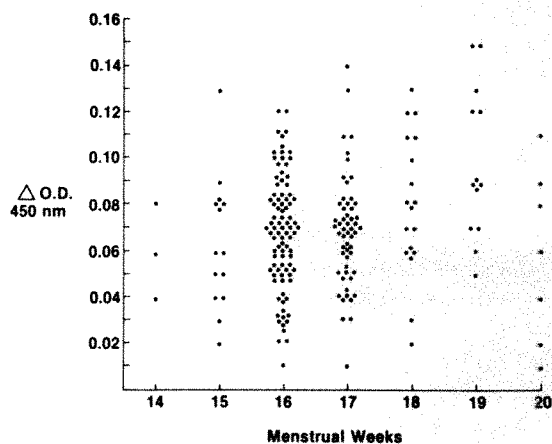


Fig. 1. Distribution of amniotic fluid ΔOD_{450} values for normal pregnancies between 14 and 20 menstrual weeks plotted against weeks of gestation (menstrual age).

either Rh-positive with no irregular antibodies or Rh-negative with no Rh or irregular antibodies were included in this study.

Each sample of amniotic fluid was centrifuged immediately at 3000 rpm for 10 minutes. Then 3 ml aliquots of supernatant fluid were transferred to a quartz cuvette and analyzed in a Perkin Elmer 202 spectrophotometer with use of a visible light source. A continuous recording was obtained between the wavelengths 300 nm and 700 nm on linear-scale graph paper, with a slit of 25 and scan speed set at "fast." The deviation in optical density was plotted by drawing an arbitrary

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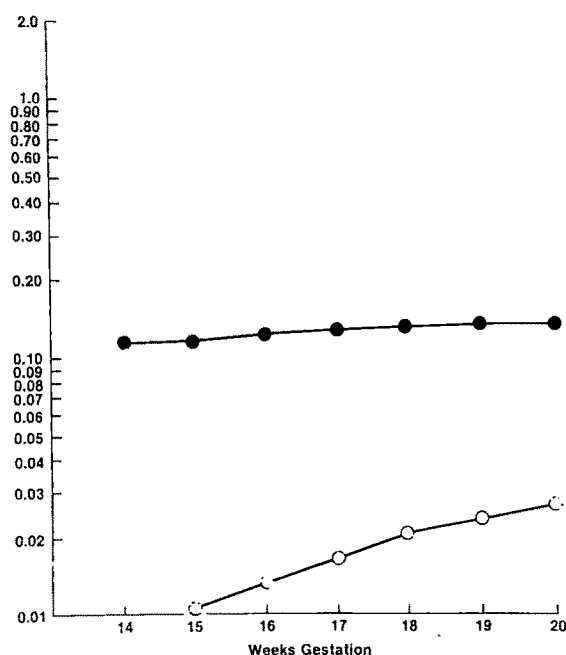


Fig. 2. Ninety-five percent confidence limits for amniotic fluid ΔOD_{450} from 14 to 20 weeks' gestation.

line from 375 nm to 525 nm and measuring the difference at 450 nm as described by Liley.¹ Twenty-four amniotic fluid samples demonstrating significant peaks at 412, 540, and 575 nm, and thus representing contamination with blood, were excluded from the study, since these peaks distort the ΔOD_{450} values. Therefore the following results were derived from 180 amniotic fluid samples, which were free of blood contamination.

Results

Fig. 1 shows the distribution of the ΔOD_{450} values plotted against the weeks since the last normal menstrual period. The lowest value is 0.01 and the highest is 0.15. Regression analysis of these data reveals a slight but statistically significant upward trend ($p = 0.03$) in ΔOD_{450} as pregnancy advances.

The 95% confidence limits for amniotic fluid ΔOD_{450} from 14 to 20 weeks' gestation in normal pregnancies are shown in Table I and Fig. 2. The *black circles* represent the upper 95% prediction levels and the *open circles* the lower ones.

Seven patients underwent a second amniocentesis from 2 to 4 weeks later because of inadequate cell growth in culture. The amniotic fluid ΔOD_{450} values are represented in Fig. 3. The second value was higher in four, lower in two, and the same in one.

Comment

The ΔOD_{450} is known to correlate well with the level of indirect bilirubin in the amniotic fluid. This study

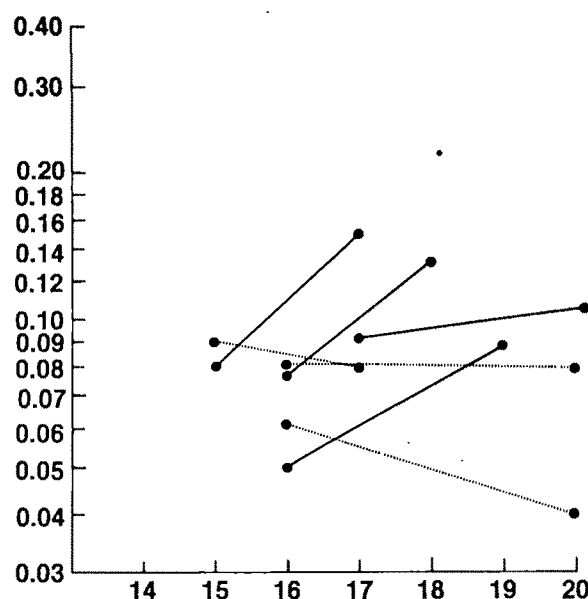


Fig. 3. Serial amniotic fluid ΔOD_{450} values in normal patients.

Table I. Ninety-five percent confidence limits for amniotic fluid ΔOD_{450}

Week of gestation	Lower 95% prediction limit on ΔOD_{450}	Upper 95% prediction limit on ΔOD_{450}
14	0.006	0.115
15	0.010	0.118
16	0.014	0.121
17	0.017	0.125
18	0.021	0.129
19	0.024	0.133
20	0.027	0.137

shows a wide range of normal values from 14 to 20 weeks' gestation, with a slight upward trend of values as pregnancy progressed.

Berk and Sussman⁴ reported on amniotic fluid ΔOD_{450} values in 54 women undergoing termination of pregnancy from 10 to 24 weeks' gestation and showed no trend with increasing gestation. Present results agree with those of Niswander,⁵ who concluded from a study of 34 patients that the bilirubin content of amniotic fluid increases from 10 to 17 weeks' gestation, remains stable to 26 weeks, and thereafter decreases to term. But Niswander did not perform statistical analysis on his very small series to corroborate these trends.

Later in pregnancy, from 22 to 26 weeks' gestation, the amniotic fluid bilirubin trend has been shown to be horizontal.⁶ From approximately 25 to 26 weeks to 36 weeks the trend is downward.^{1, 2, 6} It is this well-documented downward trend that was the basis for Liley's three-zone graph. Over the years various attempts have been made to extend the slope of the Liley graph back earlier than 27 weeks' gestation. The data presented in

this study show that this is not logical because the normal values are much lower in early pregnancy. Furthermore, this practice could be dangerous because in early gestation fetal deterioration can occur in the presence of lower values. Since patients with very severe Rh disease are still being encountered, this information is extremely important in evaluating fetal condition and in determining whether an intrauterine transfusion is indicated during the second trimester of pregnancy. By determining the normal values for amniotic fluid ΔOD_{450} , pathologically elevated values can be evaluated better.

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Adhesion prevention in the rabbit with sodium carboxymethylcellulose solutions

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With the use of a rabbit surgical model, the ability of 1%, 2%, and 3% solutions of sodium carboxymethylcellulose to reduce postoperative uterine adhesions was determined. At all concentrations sodium carboxymethylcellulose was more effective than either 32% dextran 70 or heparinized lactated Ringer's solution. Neither the dextran nor Ringer's solution had a significant ($p \leq 0.05$) effect. The beneficial effects of sodium carboxymethylcellulose were closely dose-dependent (correlation coefficient 0.97). Sodium carboxymethylcellulose is highly effective in reducing postoperative adhesions in laboratory animals, and additional studies are warranted to further define its efficacy and safety. (AM J OBSTET GYNECOL 1986;155:667-70.)

Key words: Adhesions, carboxymethylcellulose, uterus

Postoperative peritoneal adhesion formation is a potential problem in all abdominal operations and is of particular concern in the gynecologic patient. Postoperative adhesions can disrupt reproductive processes and significantly impair fertility. Surgically induced adhesions may result from several factors, including tissue ischemia,^{1,3} tissue trauma and the resulting exudation of proteinaceous fluid, improper hemostasis,⁴ and tissue reaction to foreign bodies such as suture materials or talcum powder.^{5,6} Parenteral and locally instilled medications, suture materials of low reactivity, mechanical barriers, and specialized surgical techniques have all been tried with variable degrees of success in preventing adhesions.⁷⁻⁹

Intraperitoneal instillation of various high molecular weight solutions, particularly 32% dextran 70 (Hyskon, Pharmacia, Piscataway, New Jersey), is a common practice. These solutions seem to reduce adhesion formation by several mechanisms, including creating a protective, lubricating coating between serosal and

peritoneal surfaces. Gelatinous solutions of sodium carboxymethylcellulose (Hercules Inc., Wilmington, Delaware) are more effective than dextran in preventing the initial formation and postlysis reformation of surgically induced adhesions in rats.^{10,11} The purpose of this study was to assess the effectiveness of different concentrations of sodium carboxymethylcellulose in preventing postoperative abdominal adhesions in rabbits.

Material and methods

Animals. The animals used throughout these investigations were nonpregnant adult female New Zealand White rabbits weighing 3.0 to 4.5 kg. All were housed individually, with an ambient temperature of 21° C and a regulated light cycle of 14 hours of light and 10 hours of dark. All animals were held for at least 2 weeks before study and were fed commercial rabbit chow and water ad libitum.

Surgical protocol. The reproductive tract was exposed through a midline abdominal incision, and three distinct surgical lesions were made in order to induce adhesions (Fig. 1). A three-sided rectangular flap (2 by 0.5 cm) was cut from the full thickness of one uterine horn and resutured with continuous 4-0 silk (Ethicon, Inc., Sommerville, New Jersey). A longitudinal cut (2 cm) was made through the wall of the contralateral uterine horn and also closed with 4-0 silk. Care was taken with both lesions to prevent the eversion of mu-

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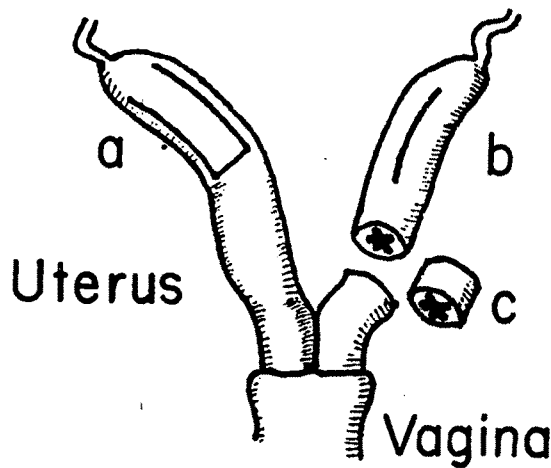


Fig. 1. Diagrammatic representation of uterine surgical model. *a* = Flap; *b* = longitudinal cut; *c* = reanastomosis. *a* and *b* were sutured with 4-0 silk; *c* was reanastomosed with 7-0 silk.

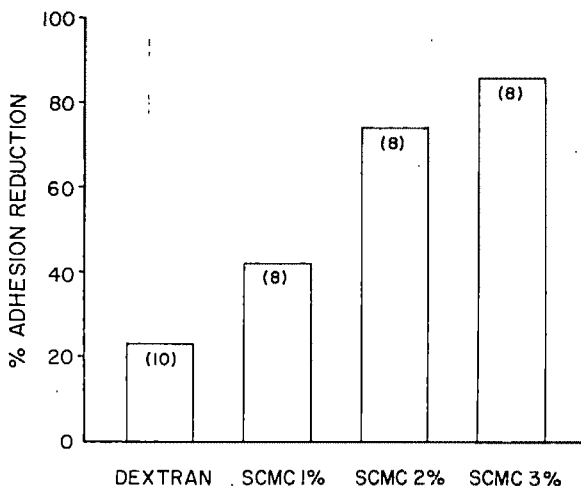


Fig. 2. Reduction in total adhesion scores in comparison with those of dry controls. Number of animals is shown in parentheses. SCMC = Sodium carboxymethylcellulose.

cosa into the sutured lesion. No effort was made to control uterine bleeding. The third lesion involved excision of a 0.5 cm segment of the uterine horn without the flap and its microsurgical reanastomosis with 7-0 silk. In this instance, hemorrhage was minimized by infiltrating a 1:10,000 epinephrine solution. The abdominal incisions were closed in three layers with 3-0 Dexon (Davis and Geck, Inc., Puerto Rico).

As preparation for operation, all animals were shaved, scrubbed, and draped. All received 200,000 units of penicillin and 250 mg of dihydrostreptomycin intramuscularly before operation. Operation was performed with maximal asepsis and at suitable levels of anesthesia induced with a 3:1 ketamine/xylazine so-

lution intramuscularly. Postoperative animal care was meticulous.

Treatment groups. Fifty-one rabbits were randomly assigned to six treatment groups: (1) dry surgical control, $n = 10$; (2) heparinized (5000 U/L) lactated Ringer's solution, 10 ml/kg intraperitoneally, $n = 7$; (3) 32% dextran 70 (Hyskon), 7 ml/kg intraperitoneally, $n = 10$; (4) sodium carboxymethylcellulose 1%, 7 ml/kg intraperitoneally, $n = 8$; (5) sodium carboxymethylcellulose 2%, 7 ml/kg intraperitoneally, $n = 8$; (6) sodium carboxymethylcellulose 3%, 7 ml/kg intraperitoneally, $n = 8$.

All solutions were instilled into the abdominal cavity through a catheter after closure of the muscular layer. The sodium carboxymethylcellulose solutions (7H 4F, food grade) were initially prepared as 1% by weight per volume of distilled water and then concentrated by water distillation. All were sterilized by immersion in boiling water for 10 minutes.

Adhesion scoring. All animals were allowed to recover for 2 weeks; the animals were then put to death, at which time a complete autopsy was performed. The extent of pelvic adhesion formation was assessed without knowledge of each animal's specific treatment. Adhesions associated with each lesion were scored separately and combined to produce a total adhesion score. Adhesions were graded as: grade 0, no adhesions; grade 1, localized, filmy adhesions; grade 2, localized, dense adhesions; grade 3, widespread, filmy adhesions; grade 4, widespread, dense adhesions.

Adhesion scores were grouped and differences between groups were tested for significance with the Mann-Whitney test. Alpha levels of $p \leq 0.05$ were considered significant.

Sodium carboxymethylcellulose. Sodium carboxymethylcellulose is a water-soluble polymer derived from cellulose, which is widely used in the food, cosmetic, and pharmaceutical industries and is commonly known as cellulose gum. Sodium carboxymethylcellulose is prepared from natural cellulose by reaction with monochloroacetate. Its molecular weight varies, reflecting the specific cellulose source from which it is prepared. Food grade forms exhibit a molecular weight of up to 350,000¹⁰ and are not metabolized.¹² Aqueous solutions of sodium carboxymethylcellulose are clear and semigelatinous. Solutions have been prepared that closely simulate the lubricating properties and viscosity of synovial fluid.¹² In limited animal tests, sodium carboxymethylcellulose injected into rabbit anterior eye chambers and knee joints was nonirritating and caused no apparent damage.¹² Dermatologic and toxicologic studies demonstrate that sodium carboxymethylcellulose shows no evidence of being toxic to white rats, dogs, guinea pigs, or human beings. Feeding, metabolism, and topical use studies also show that it is physiologically

Table I. Postoperative adhesion scores (mean $\pm$ SEM) on day 14

	Dry (n = 10)	Dextran (n = 10)	Heparinized lactated Ringer's solution (n = 7)	Sodium carboxymethylcellulose 1% (n = 8)	Sodium carboxymethylcellulose 2% (n = 8)	Sodium carboxymethylcellulose 3% (n = 8)
Longitudinal cut	0.8 $\pm$ 0.3	0.4 $\pm$ 0.4	0.7 $\pm$ 0.5	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Reanastomosis	1.0 $\pm$ 0.3	1.1 $\pm$ 0.4	1.3 $\pm$ 0.3	0.5 $\pm$ 0.3	0.3 $\pm$ 0.2	0 $\pm$ 0*
Flap	3.9 $\pm$ 0.1	2.9 $\pm$ 0.5	4.0 $\pm$ 0	2.8 $\pm$ 0.4*	1.3 $\pm$ 0.5††	0.8 $\pm$ 0.5††
All lesions	5.7 $\pm$ 0.5	4.4 $\pm$ 1.0	6.0 $\pm$ 0.6	3.3 $\pm$ 0.5‡	1.5 $\pm$ 0.4††	0.8 $\pm$ 0.5††

*p < 0.05 compared with dry control.

†p < 0.05 compared with dextran.

‡p < 0.01 compared with dry control.

Table II. Distribution of animals according to total adhesion scores on day 14

Total score	Dry	Heparinized lactated Ringers solution	Dextran	Sodium carboxymethylcellulose 1%	Sodium carboxymethylcellulose 2%	Sodium carboxymethylcellulose 3%
0	0	0	2	0	2	5
1	0	0	0	0	2	2
2	0	0	1	3	2	0
3	1	0	1	2	2	0
4	1	1	1	2	0	1
5	3	2	1	0	0	0
6	2	2	2	1	0	0
7-10	3	2	2	0	0	0

inert. Patch tests on human skin have demonstrated that sodium carboxymethylcellulose is neither a primary irritant nor a sensitizing agent. (Detailed reports are on file with the Food and Drug Administration and additional information is available from the Hercules Corporation.) Sodium carboxymethylcellulose solutions appear to be absorbed more slowly from the abdomen than solutions of dextran.^{10,11} Little is known, however, about the extent and manner in which sodium carboxymethylcellulose is excreted.

Results

A surgical model was used to assess the effectiveness of sodium carboxymethylcellulose in reducing the postoperative adhesions associated with three different types of lesions. All animals but one survived, with no evidence of infection or hemorrhage at autopsy 14 days later. This one animal, which was treated with heparinized lactated Ringer's solution, died during the recovery period and was eliminated from the study. The adhesions scores for each surgical lesion, as well as for the entire abdomen, are summarized in Table I. The ischemic uterine flap consistently produced more severe adhesions than either the reanastomosis or longitudinal cut and was the main determinant of overall scores. The most effective agent in reducing adhesion formation was sodium carboxymethylcellulose, significantly reducing adhesions at all concentrations (Table II, Fig. 2). This effect was dose-dependent, with a correlation coefficient between dose and effect of 0.97.

Neither dextran nor heparinized lactated Ringer's solution significantly ($p \leq 0.05$) reduced adhesions compared with those of dry controls.

In the sodium carboxymethylcellulose 3% group an average weight loss of 12% was noted with all other groups losing <5%.

Comment

Intraperitoneal instillation of high molecular weight solutions has been used in an effort to prevent adhesion formation after surgical trauma to abdominal viscera. Such agents are thought to provide a mechanical barrier between adjacent serosal and peritoneal surfaces thereby preventing adhesion formation. In reconstructive operations in women, the most commonly used solution is dextran 70, which is instilled almost exclusively as 32% dextran 70 in dextrose (Hyskon).^{13,14} In addition, reconstructive operation is usually accompanied by intraoperative irrigation, often with heparin-containing solutions.

Experiments involving a cecal abrasion in rats indicated that a 1% solution of sodium carboxymethylcellulose is more effective than 32% dextran 70 in preventing both the initial formation and the reformation of peritoneal adhesions.^{10,11} In the present study, 7 ml/kg of 1%, 2%, and 3% solutions of sodium carboxymethylcellulose instilled intraperitoneally after uterine operations in rabbits were more effective in preventing adhesions than either 32% dextran 70 or heparinized lactated Ringer's solution. This effect was closely dose-

related. The sodium carboxymethylcellulose volume per kilogram dose is one third to one sixth that used in the rat.^{10, 11}

While the mechanism of action of sodium carboxymethylcellulose is not known, it is highly viscous and likely to serve as a barrier and lubricant preventing the adherence of adjacent visceral surfaces. Elkins et al.¹⁰ have shown that sodium carboxymethylcellulose is absorbed from the rat peritoneal cavity more slowly than 10% dextran 40, which may account for its greater effectiveness. Sodium carboxymethylcellulose may also be less antigenic than dextran and less likely to support bacterial growth.^{11, 15, 16} Instillation of 10 ml of 32% dextran 70 into the abdomen of rats has resulted in death within 24 hours, an effect possibly mediated by an allergic response.¹¹ Instillation of a similar volume of sodium carboxymethylcellulose had no such effect. Sodium carboxymethylcellulose is highly effective in reducing postoperative adhesions in laboratory animals, and additional studies are warranted to further define its efficacy and safety.

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Effect of oxytocin on spontaneous electrical and mechanical activities in pregnant human myometrium

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Spontaneous intracellular electrical activity and contraction of pregnant human myometrium were recorded by the single sucrose-gap method, and the effects of oxytocin on the muscle were studied. In pregnant human myometrium at term, both plateau and spike types of action potentials were observed. All contractions were well synchronized with each action potential. Oxytocin, 10^{-2} U/ml, potentiated spontaneous contractions by enhancing the plateau part of action potentials; spike-type configuration became plateau. When extracellular ionized calcium was removed, spontaneous activities disappeared, while 10^{-2} U/ml of oxytocin could evoke action potentials and contractions but these were smaller than those of the controls. Spontaneous activities also disappeared when ionized calcium was increased to 5 mmol/L, but oxytocin evoked plateau potentials and contractions remarkably. Diltiazem (ionized calcium antagonist), 10^{-6} gm/ml, suppressed the spontaneous activity, but oxytocin evoked action potentials and contractions in high frequency, the duration of the action potential being short and the contraction being small. In the presence of 10^{-4} gm/ml of diltiazem, 10^{-2} U/ml of oxytocin could not evoke any action potentials but did evoke small and long contractures, while in a high ionized potassium contracture experiment, oxytocin potentiated the tonic phase. These results suggest that oxytocin can increase spontaneous contractions by enhancing plateau potentials and that this effect requires sufficient extracellular ionized calcium. In this potentiation, the effects on frequency and amplitude of contractions might vary. It is also suggested that oxytocin may evoke a contracture in the absence of an action potential by releasing calcium from intracellular storage sites. (AM J OBSTET GYNECOL 1986;155:671-6.)

Key words: Oxytocin, action potential, contraction, human myometrium

It is well known that oxytocin increases the frequency as well as the force of spontaneous contractions of the uterus. Therefore, this drug is the most readily available agent for induction and augmentation of labor in obstetric practice.

Using the intracellular microelectrode method in animal experiments, Marshall¹ has shown that oxytocin increases frequency of both spike discharges and contraction, and Osa and Taga² have described that 10^{-4} U/ml of oxytocin depolarizes the uterine cell membrane and accelerates the spike activity in a manner similar to that of carbachol. More recently, Mironneau³ observed by means of a voltage-clamp technique that oxytocin increased the calcium component of the inward current and the increased frequency of the rhythmic activity might result in an increase in contractile force by summation of the contractions. However, in human myometrium, the effects of the drug on the electrical activity at the cellular level have not yet been clarified.

In the present investigation, we studied the effects of oxytocin on the electrical and mechanical activities of pregnant human myometrium and investigated the influence of extracellular calcium ion and the calcium antagonist diltiazem.

Material and methods

Material. Small strips of pregnant human isthmus myometrium were dissected from the upper incisional edge of the lower uterine segment at the time of term cesarean section with the patient under epidural anesthesia before the administration of the oxytocics. The strips were immediately placed in cold modified Krebs solution and transported to the laboratory. Most were stored at 4° C overnight and used for experiments the next day, while some were tested within 1 hour after operation. No differences in spontaneous activity or reaction to oxytocin were found between samples used immediately and those stored overnight.

Informed oral consents were obtained from the patients beforehand.

Experimental protocol. The strip was dissected under a binocular microscope and set in a single sucrose-gap apparatus, which was composed of three compartments, each being separated by thin rubber with a tiny hole through which the muscle strip was passed.

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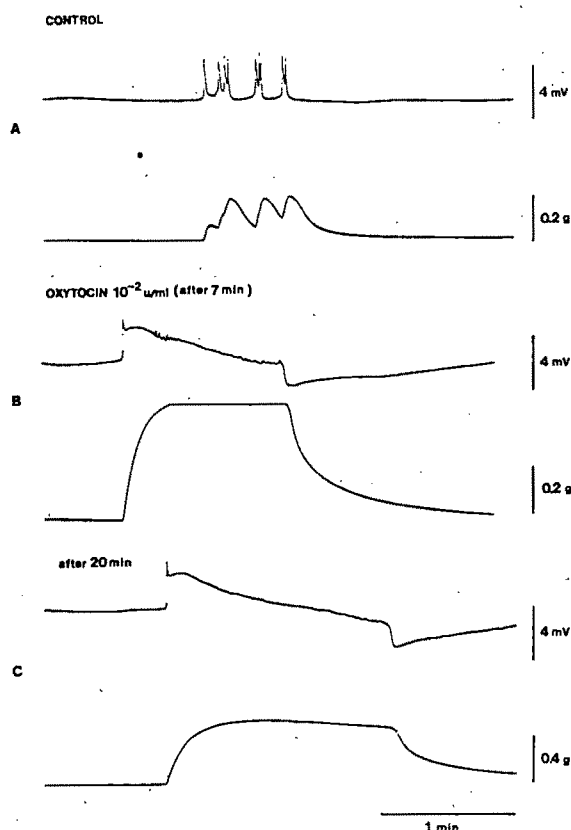


Fig. 1. Effect of 10^{-2} U/ml of oxytocin on spontaneous spike-type action potentials and contractions of human isthmus myometrium recorded by the single sucrose-gap method. Trace A shows control and B represents responses 7 minutes after the introduction of oxytocin. Contraction trace was saturated in B. Trace C shows the response after 20 minutes. The upper traces reveal the electrical activities, and the lower traces, the contractions.

The central chamber, into which the sucrose was perfused, was 1 mm wide, and one side of the three compartments was filled with isotonic potassium chloride, while the other side was perfused by the test solution. Muscle strips were spontaneously active by this method. For contraction experiments, strips 1 to 1.5 mm in diameter and 4 to 5 mm in length were cut longitudinal to the small muscle bundles under a binocular microscope, suspended vertically in a 1 ml chamber, and prepared for recording of isometric contractions. Potassium contracture was induced by means of a solution in which the sodium of the modified Krebs solution was replaced by equivalent amounts of potassium. Each experiment was started after the muscle began to contract spontaneously and showed stable rhythmic contractions.

Modified Krebs solution was used in these experiments; the ionic composition was as follows: ionized sodium, 137.4 mmol/L; ionized potassium, 5.9 mmol/L; ionized magnesium, 0.3 mmol/L; ionized cal-

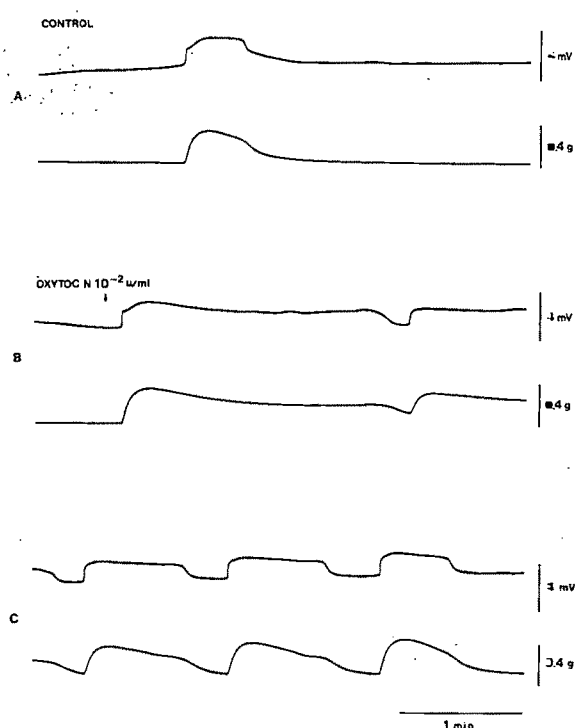


Fig. 2. Effect of 10^{-2} U/ml of oxytocin on spontaneous plateau-type action potentials and contractions. A, Control. B and C are successive records representing the responses of oxytocin. The electrical (upper traces) and mechanical (lower traces) activities were recorded simultaneously.

cium, 2.5 mmol/L; chlorine, 134.0 mmol/L; bicarbonate, 15.5 mmol/L; H_2PO_4^- , 1.2 mmol/L; and glucose, 11.5 mmol/L. As the frequency of spontaneous contraction was affected by the external ionized magnesium concentration as reported previously,⁴ ionized magnesium was reduced from 1.2 to 0.3 mmol/L to obtain more frequent contractions. The solution was equilibrated with a mixture of 97% oxygen and 3% carbon dioxide. The standard bathing temperature was between 34° and 36° C.

Oxytocin (Teikokuzoki Pharm. Co. Ltd.) and diltiazem (Tanabe Pharm. Co. Ltd.) were used and the concentrations were given as units per milliliter and grams per milliliter, respectively, in the results. The drugs were added to the perfusing solution.

Results

Effect of oxytocin on spontaneous electrical and mechanical activities in human isthmus myometrium.

In pregnant human isthmus myometrium from term cases, spike-type and plateau-type action potentials were observed; the latter was more frequently observed, as described previously.⁵ Fig. 1 shows spike-type action potentials and the effect of oxytocin on them. Fig. 1, A, shows a control in which spike potentials were prominent and contraction was well synchronized with

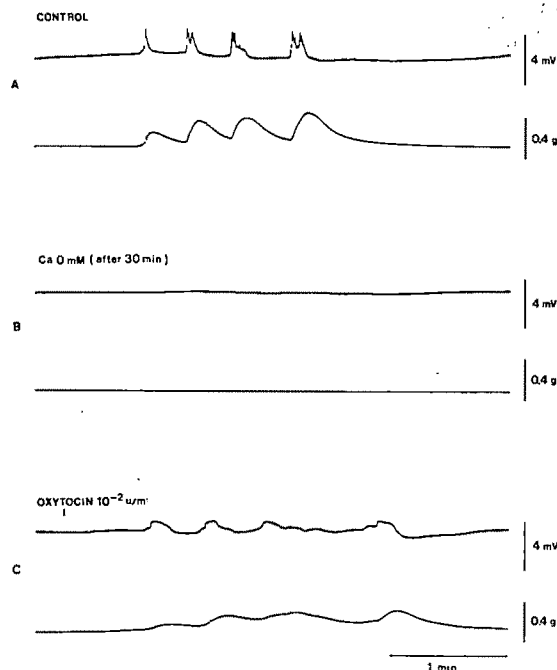


Fig. 3. Effect of 10^{-2} U/ml of oxytocin on spontaneous activities after removal of external calcium. *A*, Control. *B*, Removal of ionized calcium. Trace *C* shows the effect of oxytocin in external ionized calcium, 0 mmol/L. *Upper traces* represent the electrical activities, and the *lower traces*, the contractions.

each spike potential. When 10^{-2} U/ml of oxytocin was applied, the action potential pattern changed to the typical plateau type, as shown in Fig. 1, *B* and *C*. In Fig. 1, *B*, small abortive spikes remained on the plateau, but they were then abolished and the plateau duration was more prolonged in Fig. 1, *C*. Amplitudes of the initial spikes and the plateau potentials were not altered; however, after a change to Krebs solution, the pattern returned to the spike-type action potential. The contractions were potentiated in proportion to the changes in the action potential (Fig. 1, *B* and *C*).

Fig. 2 shows plateau-type action potentials and the effects of oxytocin on them, with Fig. 2, *A*, being a control. Contraction was well synchronized with the plateau potential. When 10^{-2} U/ml of oxytocin was added to the perfusing solution, the plateau was enhanced and the duration of the action potential was lengthened, thus increasing the frequency, as shown in Fig. 2, *B* and *C*. The contraction was potentiated in proportion to changes in the electrical activity.

Effect of oxytocin on spontaneous electrical and mechanical activities under low or high external calcium conditions. Fig. 3 shows the effect of oxytocin on spontaneous activities of human isthmus myometrium under low external calcium. Fig. 3, *A*, depicts a control in which small abortive spikes superimposed on the small plateau were observed. Contractions were well

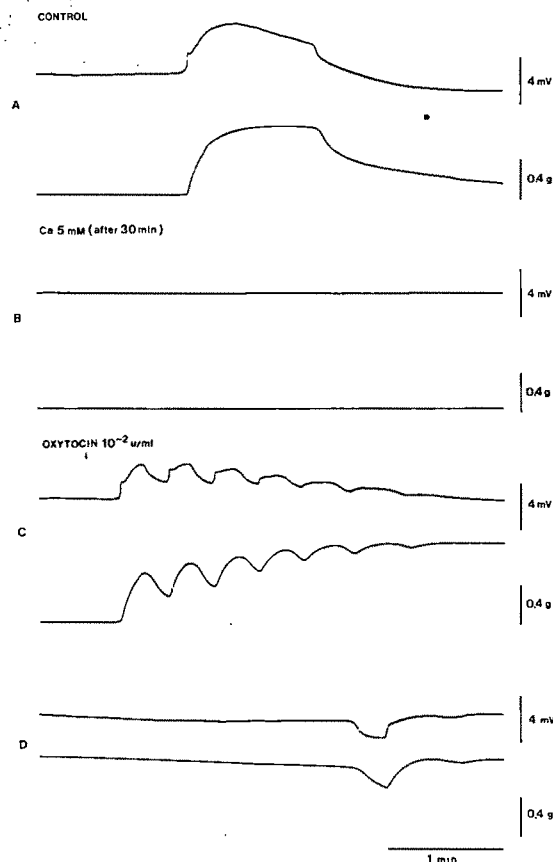


Fig. 4. Effect of 10^{-2} U/ml of oxytocin on spontaneous electrical (*upper traces*) and mechanical (*lower traces*) activities in ionized calcium, 5 mmol/L. *A*, Control. *B*, Traces in ionized calcium, 5 mmol/L. *C* and *D* are continuous records representing the responses of oxytocin in ionized calcium solution, 5 mmol/L.

synchronized with the action potentials. When external calcium was decreased from 2.5 mmol/L in Krebs solution to 0 mmol/L, spontaneous activities disappeared, as shown in Fig. 3, *B*. Oxytocin, 10^{-2} U/ml, could evoke activities under this condition; however, the evoked plateau potentials and corresponding contractions were small (Fig. 3, *C*).

Fig. 4 shows the effect of oxytocin under conditions of high calcium, with Fig. 4, *A*, being a control. Plateau potential was prominent in this specimen and contraction was synchronized with the plateau potential as well. When external calcium was increased from 2.5 mmol/L (with Krebs solution) to 5 mmol/L, spontaneous activities also disappeared (Fig. 4, *B*). In 5 mmol/L calcium solution, 10^{-2} U/ml of oxytocin was added, and then spontaneous plateau potentials appeared and were markedly potentiated as shown in Fig. 4, *C* and *D*. Accompanying contractions were also greatly increased.

Effect of oxytocin in the presence of the calcium antagonist diltiazem. Fig. 5 shows the effect of oxytocin

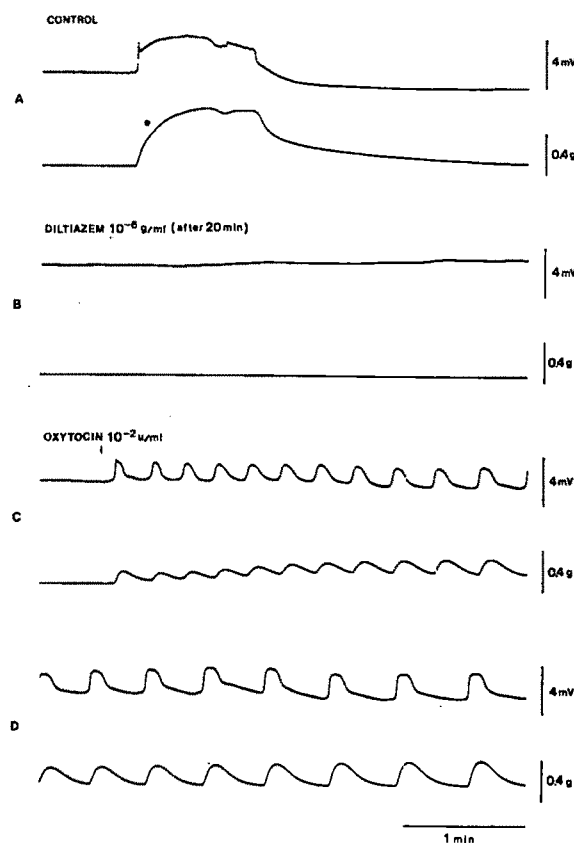


Fig. 5. Effect of 10^{-2} U/ml of oxytocin on spontaneous activities in the presence of 10^{-6} gm/ml of diltiazem. Trace A is a control, and B shows the responses to application of 10^{-6} gm/ml of diltiazem. C and D are continuous traces showing the effect of oxytocin in the presence of diltiazem. The upper traces show electrical activities, and the lower ones, the contractions.

on spontaneous activities in the presence of 10^{-6} gm/ml of diltiazem. The control action potential was composed of an initial spike followed by a long plateau potential, as shown in Fig. 5, A. The contraction was well synchronized with the action potential. The spontaneous activities were completely abolished by the application of 10^{-6} gm/ml of diltiazem (Fig. 5, B); however, 10^{-2} U/ml of oxytocin could evoke the activities, as shown in Fig. 5, C and D. The evoked action potentials showed small and frequent plateau potentials and the corresponding contractions were also frequent but small.

Fig. 6 shows the effect of the same dose of oxytocin in the presence of 10^{-4} gm/ml of diltiazem, with Fig. 6, A, being a control for the plateau-type action potential. When 10^{-4} gm/ml of diltiazem was applied, spontaneous activities were abolished (Fig. 6, B). In this condition, 10^{-2} U/ml of oxytocin induced a small tonic contraction with no electrical activities, as shown in Fig. 6, C.

Effect of oxytocin on potassium contracture. In hu-

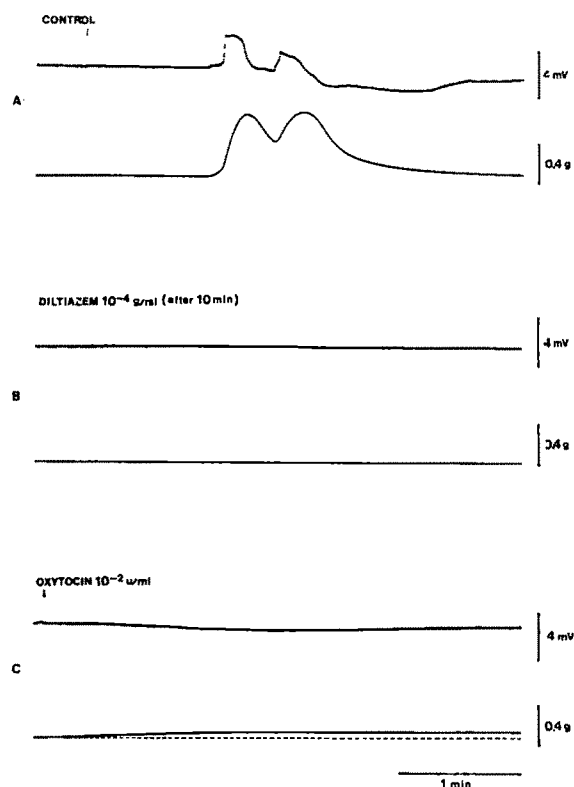


Fig. 6. Effect of 10^{-2} U/ml of oxytocin on spontaneous electrical (upper traces) and mechanical (lower traces) activities in the presence of 10^{-4} gm/ml of diltiazem. A, Control. B, 10^{-4} gm/ml of diltiazem. C, Represents the effect of oxytocin in the presence of the same dose of diltiazem as in B.

man myometrium, high ionized potassium contracture revealed a phasic contraction followed by tonic phases and another phasic contraction during the recovery process, as shown in Fig. 7, A. When 10^{-2} U/ml of oxytocin was added at the tonic phase, the muscle potentiated the contraction (Fig. 7, B).

Comment

In human myometrium, oxytocin increased the amplitude and duration of spontaneous contractions in both spike-type and plateau-type action potentials but affected the frequency to a lesser degree, as reported previously.⁶ As for the effect on the action potential, the drug enhanced the plateau potential in the plateau type and changed spike-type action potentials into plateau ones. In the present experiments, we could generally confirm the effects of oxytocin by recording larger fine traces of electrical activities, as shown in Figs. 1 and 2. This effect on electrical activities, that is, plateau enhancement, was very consistent. We then considered this effect of oxytocin as a control and observed changes in the effect under low and high external calcium conditions, on the basis of results described previously in which plateau potential was strongly affected

by external calcium in pregnant rats.^{7,8} When external calcium was changed from 2.5 mmol/L in Krebs solution to 0 or 5 mmol/L, spontaneous electrical and mechanical activities disappeared in both solutions (Figs. 3, B, and 4, B). The mechanisms of these two types of suppressions caused by low and high external calcium might differ. For example, high calcium might suppress spontaneous activities by membrane stabilization, while, on the other hand, low calcium might decrease the influx of calcium and consequently suppress the activities. The effects of external calcium on spontaneous activities of human myometrium were discussed in detail in our previous article.⁹ Under these conditions, oxytocin evoked plateau potentials and accompanying contractions; however, the activities were small in 0 mmol/L of calcium and markedly larger in 5 mmol/L (see Figs. 3 and 4). In animal experiments, it is postulated that the response of the uterus to neurohypophyseal hormones, such as oxytocin, is calcium dependent.¹⁰ In human myometrium, it is also suggested that the stimulatory effect of oxytocin needs calcium ion and depends on the external calcium concentration.

Furthermore, the effect of oxytocin in the presence of the calcium antagonist diltiazem was investigated. When 10^{-6} gm/ml of diltiazem was applied, spontaneous activities were abolished (Fig. 5, B). Oxytocin, 10^{-2} U/ml, evoked frequent plateau potentials and corresponding contractions; however, the duration of each plateau was remarkably short and the amplitude of each contraction was small, as shown in Fig. 5. This effect, that is, small and frequent plateau potentials and contractions, was characteristic and observed in the spike-type action potential. Accordingly, the excitatory effect of oxytocin on frequency and amplitude of spontaneous contractions may be affected by the duration of plateau potentials, which is greatly influenced by the external calcium concentration. The dose of diltiazem was then increased to 10^{-4} gm/ml and the effect of oxytocin was observed (Fig. 6). The same dose of oxytocin could not evoke any action potentials but could stimulate a long small contraction (Fig. 6, C). With the sucrose-gap experiment, the resting membrane potential cannot be measured; therefore, the precise mechanism of this effect cannot be discussed. In pregnant rat myometrium, the membrane was remarkably depolarized in isotonic potassium solution when the membrane potential was measured after 20 to 50 minutes in test solution.¹¹ Accordingly, we performed high-potassium contracture experiments to ascertain whether oxytocin could potentiate the contraction in the state of depolarization of the membrane. Consequently, oxytocin revealed obvious potentiation of the tonic phase in high ionized potassium contracture, as shown in Fig. 7, B. These results suggest the possibility that oxytocin could release calcium ions from intracellular store sites

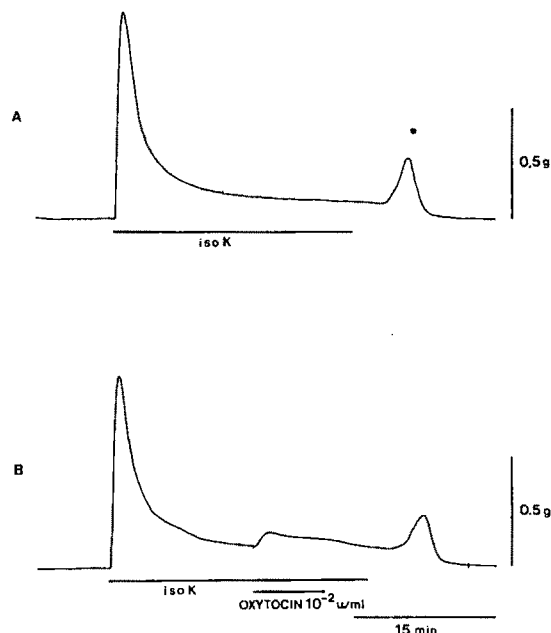


Fig. 7. Effect of 10^{-2} U/ml of oxytocin on tonic phase of high ionized potassium contracture. Trace A is a control, and B shows the effect on contracture. Isotonic potassium solution and oxytocin were added during the periods indicated by the bars.

in pregnant human myometrium, as mentioned in a review of animals.¹⁰

In obstetric practice, the regulation of frequency or amplitude of spontaneous contractions by use of oxytocics is one of the critical factors of management. In the present article, we have demonstrated the effects of oxytocin on spontaneous electrical activity and the accompanying contraction in relation to external calcium concentration. Moreover, we have pointed out the influence of the duration of plateau potential on frequency and amplitude of contractions in human myometrium. More precise investigation on excitation-contraction relation in human myometrium should be performed in the future to understand and to manage uterine contractions in clinical practice.

We are grateful to Prof. H. Nakano, Kyushu University, for his support and valuable advice in this work.

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Uterine fluid and prolactin secretion in the ovulating cynomolgus monkey

Yu-Kang Ying, M.D., John F. Randolph, M.D., Donald B. Maier, M.D., Sally T. Kuslis, Jane Chapitis, M.D., and Daniel H. Riddick, M.D., Ph.D.

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Immunoreactive prolactin is produced by late secretory human endometrium in vitro. Human myometrium in explant culture also produces prolactin. A primate model with the use of the cynomolgus monkey is described that allowed repeated samplings of uterine secretions in vivo. The uterine secretory prolactin thus measured appears immunoreactively similar to human serum prolactin, and the pattern of secretions reflects the previously described pattern of endometrial prolactin production in vitro. (AM J OBSTET GYNECOL 1986;155:677-80.)

Key words: Uterine secretion, prolactin

The orderly maturation of human endometrium is not only associated with characteristic histologic decidual changes but also accompanied by de novo synthesis and release of endometrial prolactin.¹ Immunoreactive prolactin is detectable on day 23 of an idealized 28-day menstrual cycle in endometrial explant culture and increases steadily with increasing decidualization.² With endometrial prolactin production used as a biochemical marker, pathologic conditions such as luteal phase defect and corrected luteal phase defect have been studied.^{2,3} Human myometrium has also been shown to be capable of producing prolactin in vitro.⁴

For technical reasons most studies on uterine proteins have been performed on subprimates. Although the production of prolactin by human endometrium and myometrium is documented in vitro, similar data in vivo are not available. Repeated measurements of physiologic and altered physiologic states have not been attempted.

Cynomolgus monkeys have gained popularity as a primate model for the study of the hypothalamic-pituitary-ovarian axis. The uterus as an end organ has attracted less attention. We have attempted to establish a primate model for repeated measurements of uterine secretions with the use of the cynomolgus monkey.

Three cynomolgus monkeys were studied during 11 ovulatory cycles to establish this model.

Method

Uterocutaneous fistula. A chronic uterocutaneous fistula was surgically prepared for repeated samplings (Fig. 1).^{5,6} The female monkey was anesthetized with ketamine, acepromazine, and atropine. The lower abdomen was surgically opened, and the uterine fundus held with clamps. A cork borer of 8 mm inside diameter was inserted through the uterine fundus anteriorly until the uterine cavity was visible to remove a core of myometrium. The anterior peritoneum was closed circumferentially around the uterine opening, thus inverting the peritoneum into the myometrium. The fascia of the anterior rectus sheath was similarly sutured into the myometrium. Both peritoneum and rectus sheath now support the uterus so that the fundus lies at the level of the skin incision. The skin incision was then closed. After the formation of a uterocutaneous fistula, the fistula was sounded twice weekly with a uterine probe. Healing occurred readily, leaving a fistulous tract with an inconspicuous abdominal opening. Infections were not observed after the initial inflammation subsided in 3 to 4 weeks. Experiments were then begun.

Uterine fluid sampling. Monkeys were anesthetized for fluid sampling and/or venipuncture with 0.3 to 0.4 ml of ketamine. Samplings were performed in the morning between 9 and 12 AM for the duration of the study. For each venipuncture 2 ml of blood was obtained by femoral stick. Fluid aspirations were achieved via an Intramedic (Fisher Scientific Co.) polyethylene tubing of 0.76 mm inside diameter. The tubing was fed through the uterocutaneous fistula, and fluid was gently aspirated. The catheter was then removed and clamped to prevent evaporation of aspirated fluid. It

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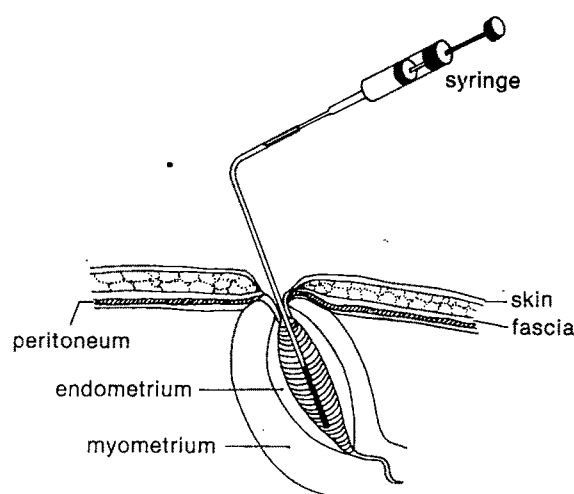


Fig. 1. Uterocutaneous fistula established for uterine fluid collection.

had been determined by control experiments that a catheter thus clamped did not lose fluid by evaporation during 4 to 6 hours. To ensure the reproducibility of volume thus aspirated, experiments were carried out comparing the direct fluid volume measurement with that calculated by a methylene blue dilution technique. The volume measured directly was determined by the formula $V = L\pi r^2$, where V = volume, L = length of tubing containing fluid, and r = inner tube radius. The methylene blue dilution allowed calculation of actual volume by diluting a given amount of methylene blue in the uterine fluid before aspiration. A standard dilution of methylene blue was injected via a catheter into the uterus, and the optical density of fluid aspirated was measured with a Perkin Elmer (Model $\lambda 3$) spectrophotometer at 668 μ . The dilution factor was then determined from a standard curve constructed by serial dilutions of methylene blue. The intra-assay variation of the optical density measurement was 2.7%. The Pearson correlation coefficient between direct fluid measurement and calculated actual fluid volume was determined for 15 samples. The correlation coefficient was found to be $r = 0.7238$ with $t = 3.7823$ and $p < 0.005$, thus demonstrating a good correlation between direct measurement and actual uterine volume by methylene blue determinations.

Radioimmunoassay. The uterine fluid prolactin concentration was measured by a standard double-antibody radioimmunoassay described by Sinha et al.⁷ Radioactive human prolactin was purchased from New England Nuclear, Boston, Massachusetts. Prolactin antibody was provided by the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health (No. AFP-C11580), Bethesda, Maryland. The

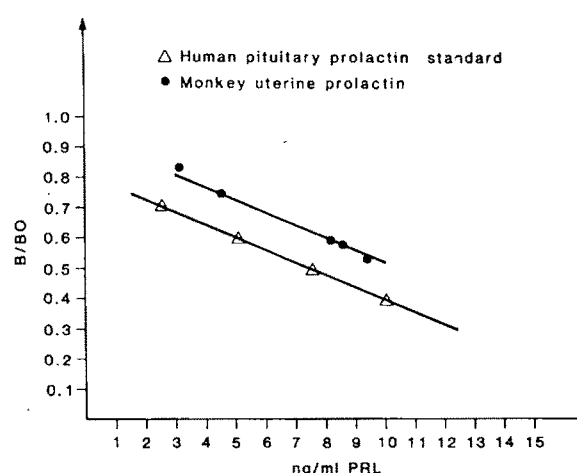


Fig. 2. Comparison of immunoreactivity of human pituitary and monkey uterine prolactin. The curves of human pituitary and monkey uterine prolactin are parallel.

immunotitration curve of the monkey prolactin from uterine secretion was found to be parallel to human prolactin (Fig. 2). Measurements of uterine prolactin of <2 ng/ml were defined as undetectable since the regression equation of serial dilutions of prolactin in nanograms per milliliter meets the y axis at $a = 1.7E$. The intra-assay and interassay variabilities were 7% and 9.3%, respectively. The 17β -estradiol concentration was measured by an immunodirect estradiol iodine-125 kit (Pantex Division of Bio-Analysis Inc., Santa Monica, CA 90404). The titration curve of monkey serum 17β -estradiol was found to be identical in immunoreactivity to human serum 17β -estradiol. The intra-assay and interassay variabilities were 2.9% and 4.4%, respectively.

The progesterone concentration was measured by the Coat-A-Count progesterone kit (Diagnostic Products Corporation, Los Angeles, CA 90045). The titration curve of monkey serum progesterone was found to be identical in immunoreactivity to human serum progesterone. The intra-assay and interassay variabilities were 5.2% and 6%, respectively.

Results

The composite pattern of volumes of uterine fluid secretions during five ovulatory cycles is shown in Fig. 3. With cessation of menstrual flow there is a sharp drop of uterine fluid volume in the follicular phase. There is an apparent increase of uterine fluid volume in the late luteal phase.

The composite pattern of uterine secretory prolactin levels and circulating levels of serum 17β -estradiol and progesterone during four ovulatory cycles is shown in Fig. 4. The 17β -estradiol and progesterone measurements confirm ovulatory menstrual cycles. The uterine

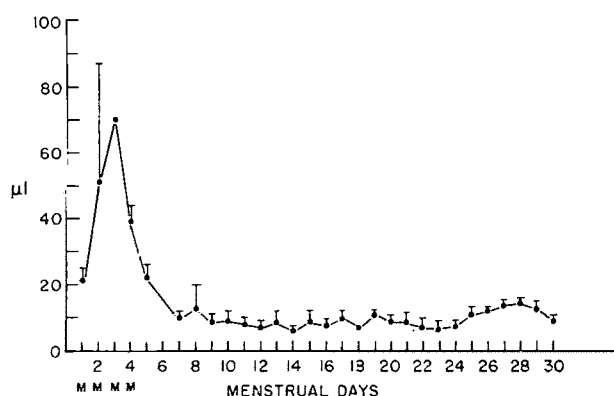


Fig. 3. Composite pattern of uterine fluid volumes during five ovulatory cycles. Vertical bars at each point indicate 1 SEM.

secretory prolactin is in general nondetectable except in the late follicular phase and the late luteal phase. The late secretory prolactin becomes detectable on day 24 and shows a dramatic increase to a mean level of 210 ng/ml on the last day of menstrual cycle. This high concentration then drops to nondetectable levels with the onset of menses.

Comment

We have described a method for repeated measurements of uterine secretions from cynomolgus monkeys. With serum 17β -estradiol and progesterone used as a guide to ovulation, there is a small but detectable rise in monkey uterine prolactin secretion in the late follicular phase concomitant with the rise in serum 17β -estradiol. Uterine prolactin then becomes nondetectable with the rise of serum progesterone at midcycle. There is then a dramatic increase of uterine prolactin secretion in the late luteal phase, which appears to coincide with a small increase in uterine fluid volume.

It is well established that estrogen and progesterone are essential for endometrial proliferation, decidualization, and maturation.⁸ Immunoreactive prolactin is detectable on day 23 of an idealized 28-day human menstrual cycle *in vitro*. There is then an increase of prolactin with increasing decidualization.² The *in vivo* concentration of prolactin in uterine fluid of the cynomolgus monkey reflects a similar pattern of production. Furthermore, it demonstrates that the prolactin produced by endometrial cells is probably secreted into the uterine fluid, since uterine levels showed very different patterns than serum levels previously described.⁹ Proliferative phase endometrium will not produce prolactin *in vitro* without an addition of progesterone.¹⁰ Moreover, increasing the estrogen/progesterone ratio will diminish the prolactin production by endometrium *in vitro*, thus demonstrating opposite effects of estrogen and progesterone on endometrial prolactin pro-

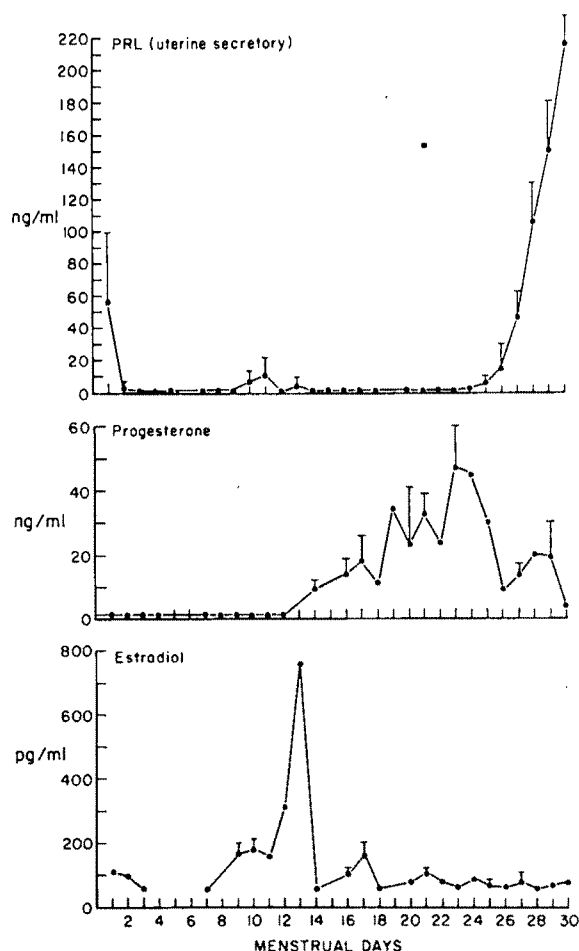


Fig. 4. Composite pattern of uterine secretory prolactin levels and circulating levels of progesterone and 17β -estradiol during four ovulatory cycles by one representative monkey. Vertical bars at each point indicate 1 SEM.

duction.¹⁰ Whereas progesterone stimulates endometrial prolactin production estrogen inhibits it.

Myometrium *in vitro* is also capable of producing prolactin. The hormonal influence on myometrial prolactin production is different from the hormone requirements for the endometrium. Estrogen tends to increase myometrial prolactin production while progesterone has the opposite effect.⁴ Thus in the cynomolgus monkey the small rise in *in vivo* uterine prolactin detected in the late follicular phase could represent myometrial prolactin, since this prolactin was noted with an increase in serum estrogen and then disappeared with an increase in serum progesterone. The late secretory prolactin probably represented endometrial prolactin since our data paralleled the *in vitro* human endometrial prolactin data in both the occurrence and time course of prolactin production in the late secretory phase.

We conclude that the *in vivo* data for uterine pro-

lactin secretion in the cynomolgus monkey are consistent with those previously reported in vitro for human endometrial and myometrial prolactin production. These data also support our previous observation³ that uterine prolactin is a useful biochemical marker for studying endometrial maturation.

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Human chorionic gonadotropin assay sensitivity on screening for ectopic pregnancy

To the Editors:

Romero et al. report an application of a radioimmunoassay of human chorionic gonadotropin (hCG) as a screening test for ectopic pregnancy (The effect of different human chorionic gonadotropin assay sensitivity on screening for ectopic pregnancy *AM J OBSTET GYNECOL* 1985;153:72-4). The type (monoclonal versus polyclonal), affinity constant, and source of the antibody are not mentioned. Their dialogue included one false negative report in 184 ectopic pregnancies but no information is supplied for false positives. With an hCG quantitative test sensitive to 10 mIU/ml, we would detect many false positives. The use of a polyclonal antibody will sometimes measure this level of hCG in tumor or even in normal tissue extract.¹ There is a large series of ectopic pregnancies where the presence of hCG could not be detected. Under these circumstances, even elaborate quantitative hCG values by the β -subunit radioimmunoassay, radioreceptor assays, or sandwich-double monoclonal techniques have failed to detect the presence of any type of pregnancy. We are surprised that the Yale team uses hCG radioimmunoassay and not a β -subunit type of test. Any luteinizing hormone (LH) surge or even low levels of LH, 2.4 mIU/ml, could very well interfere with the determination. Among the current pregnancy tests, enzyme-linked immunosorbent assay (ELISA) with use of monoclonal antibodies is relatively accurate and convenient, especially in an emergency situation, for example, an ectopic pregnancy. The ELISA technique employs two monoclonal antibodies recognizing different specific sites on the hCG molecule. ELISA, therefore, is more specific and has a sensitivity of 40 mIU/ml. The ELISA thus recognizes the intact hCG molecule and gives an index of biologic activity of the hormone. Because of the specificity of the antibody there is no interference with LH. The simplicity of these double monoclonal reactions permits a resident to perform a color test in 3 minutes and precludes the need to send a specimen to the laboratory and wait hours for the result from a hospital laboratory.

The Romero team states that less specific techniques such as the radioreceptor assay have been used; a 6% false negative rate has been reported. The receptor assay provides a measure of the biologic activity of hCG. The quantitative receptor assay can be accurate to 1 mIU of hCG. The sensitivity of the Biocept-G (radioreceptor assay) has been adjusted to 200 mIU/ml to avoid the remote possibility of interference with LH if in an extremely rare situation a woman ovulates at the time of a missed period. During pregnancy LH remains at a low level of 2 to 3 mIU/ml. In case of the possibility of a delayed LH peak, the receptor assay can be repeated in 24 to 48 hours to discriminate declining LH

levels from rising hCG levels. For example, LH surge declines to basal level where the hCG levels will double every 1.7 days up to the tenth to twelfth week of gestation.^{2,3}

We respectfully submit to our fellow clinicians that to discriminate hCG as a chemical in a solution is not to be compared to the hCG in the circulation as a marker for pregnancy. The development of specific polyclonal and monoclonal antibodies as well as the availability of specific receptor has made it possible to develop competitive binding protein assays to accurately measure as little as 1 mIU of hCG.⁴ It should be borne in mind that the levels of hCG at the time of implantation are in normal pregnancy approximately 51 mIU/ml.⁵ Hence a method that measures less than 50 mIU/ml of hCG, only confirms the production of hCG prior to or at the time of implantation, since the blastocyst also produces hCG-like material^{6,7} and does not discriminate between normal or abnormal implantation. The use of more sensitive tests is important in the subsequently declining levels of hCG, which may be the result of a blighted ova syndrome, spontaneous abortion, or missed abortion. In case of an ectopic pregnancy the hCG levels are more or less following the normal pattern in the early stages. Depending on the site of ectopic implantation, the hCG value shows subnormal increases in the repeat serum sample, so it is mandatory to repeat the pregnancy test in cases of high-risk patients for ectopic pregnancy or suspected ectopic pregnancy with close clinical correlation of symptoms. The foregoing makes it obligatory for serum hCG levels of >50 mIU/ml of hCG to be corroborated with clinical circumstances to establish a state of normal gestation. It has been amply demonstrated that even in a high-risk pregnancy destined to be ablated, the level of hCG at the time of implantation is the same as in a normal pregnancy which is destined to reach normal maturity. The name of the game is to catch the declining levels or subnormal increase of hCG during the period intervening from the time of implantation until after the missed period by repeat assays (hCG levels at missed period is <200 mIU/ml). Of course, we need the most sensitive but also specific test to measure intact hCG molecules. The β -subunits of hCG produce relatively more specific antibody, but it is biologically inert when not linked with the α -subunit. Notwithstanding highly reliable monoclonal two-site capture (sandwich-type enzyme assays) and highly immunologically specific radioassays using antibodies to specific β -subunit has provided confusion in the establishment of early pregnancy. Current sensitive radioimmunoassay tests for hCG have not been validated quantitatively by comparison with a radioligand receptor assay of hCG which measures the biologically active form of hCG. Hence the sensitivity of an immunologic test of hCG could possibly be also due to the discordant production of

hCG and its free subunits, especially in the case of an abnormal and thereby a high-risk pregnancy.⁸ This, in our opinion, is the reason for false results in so-called more improved and highly sensitive radioimmunoassays. In a molecule-like hCG the immunological specificity is conformation dependent. Even antibodies from monoclonal hybridomas may recognize epitopes that have no bearing on the specific biologic activity of hCG.

In summary, most of the current pregnancy tests have a high degree of reliability at the time of a missed period. However, for the purposes of diagnosis and management of ectopic pregnancy, repeat determinations of hCG by the aid of a reliable test and more importantly a clinical judgment are essential ingredients to control this morbidity, which is as high as one in 40 women. Finally, it is obligatory for the manufacturers of pregnancy tests not only to provide data comparing the detection of pregnancy but also to show that these tests have high correlation with radioligand receptor assay for the measurement of hCG. It is advisable to use a kit with a sensitivity >50 mIU because this level of hCG is obtained at the time of implantation. The hCG tests with greater sensitivity are suited to the monitoring of declining levels of hCG after rupture of ectopic pregnancy, spontaneous and induced abortion, chemotherapy, or surgical ablation of hCG-producing carcinoma.

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Reply

To the Editors:

While we shall endeavor to address the comments made in the letter of Landesman et al., it must be said at the outset that we fail to see exactly what point(s) these authors were trying to make, since their letter struck us as a melee of disjointed observations (some true, some false), largely disconnected from the point of the article at which the letter was directed. For example, the remarks regarding the properties and uses of the Biocept-G assay which echo what was written about this assay over 10 years ago hardly need saying and warrant no reply. The remarks about the detectability of hCG in various malignant conditions in a letter about a paper on ectopic pregnancy surely fall into the same category. What puzzled us even more, however, was the authors' allusion to the fact that serial hCG determinations can be used to evaluate further the significance of a positive hCG assay result, since, to the best of our knowledge, our group was the first to describe in detail how serial hCG measurements and grayscale sonography can be combined to the best advantage in evaluating patients for a suspected ectopic pregnancy.¹⁻⁷ Therefore we cannot reply to these authors in the coherent manner we would have liked and can only comment in a piecemeal fashion on the points they have chosen to air.

1. We use an antibody (prepared in our own laboratory some 10 years ago) raised in rabbits to a specific β -subunit (<1% α -subunit in the antigen), as clearly stated in our original paper,¹ which was referenced in the article under discussion. The referees of that original manuscript insisted, somewhat pedantically, that we desist from referring to the substance being measured in our assay as " β -hCG," since it is the intact hCG molecule in the circulation that is being measured by the antibody, even though this is by way of its β -subunit (since there are vanishingly small amounts of free β -subunit in circulation). Therefore, we have consistently referred to the substance measured in our assay as "hCG." However, in our opinion, our publications have left no room for doubt about what type of antibody is used in our laboratory. Certainly, to our knowledge, no one other than Dr. Landesman and his colleagues have ever been under the misimpression that we use an "hCG radioimmunoassay and not a β -subunit type of test." Had the authors read our papers with care, they would have spared themselves this misunderstanding and us a dissertation about how hCG antibodies that are not β -subunit-specific cross-react with luteinizing hormone; hardly news to us or your readers.

2. We are fully aware of the clinical potential of the ELISA technique as reported by Professor Chard's group from St. Bartholomew's Hospital in London,⁸ and it may well be that radioimmunoassays will become a thing of the past regarding "emergency" pregnancy testing. However, these techniques need to be evaluated in the specific context under discussion, and much more work is needed before we can determine the reliability/efficacy of serial hCG measurements with this

technique, which requires quantitation of the colorimetric response. These studies are currently being planned in our department. Perhaps we can persuade Dr. Landesman and his colleagues to turn their talents in this new direction rather than remaining stalwart champions of the radioreceptor assay which, as a pregnancy test for use in an emergency setting, clearly represents the worst of both possible worlds, since it is relatively insensitive yet requires a counter and laboratory expertise.

3. The serum hCG does not "double every 1.7 days up to the tenth to twelfth week of gestation" as the authors state. Conversely, the rate of hCG increase falls off after 6 weeks of amenorrhea.^{9,10} Recently Pittaway et al.¹¹ suggested that the log hCG-time relationship in early pregnancy is not linear at all, but quadratic. We have elsewhere expressed our criticisms of this study's design and analysis¹² and have been unable to duplicate these authors' findings. (Kadar N, Romero R. Observations on the log-hCG-time relationship in early pregnancy and their practical implications. Unpublished observations.)

4. Scrutiny of the figures in the paper by Mishell et al.,¹³ for example, clearly shows that hCG levels at the expected time of implantation following spontaneous conception can be much lower than 50 mIU/ml.

5. "There is a large series of ectopic pregnancies where the presence of hCG could not be detected" is a statement that was not referenced and is one that is completely at variance with our own quite considerable experience, as well as that reported in every publication we have read. The highest reported false negative rate for a sensitive β -subunit radioimmunoassay (10 IU/L) that we are aware of was by Sandvei et al.,¹⁴ who reported three negative tests in 100 women with ectopic pregnancies. We would certainly not accept this statement as accurate without having the opportunity to assess the data on which it was based. Negative assays in association with an ectopic pregnancy in our view signify nothing more than that hCG production by the trophoblast has ceased subsequent to its demise and that the circulation pool of hCG has been cleared by the kidneys by the time blood is drawn for analysis.

6. Finally, the authors broached three related issues, namely, what the false positive rate for our assay is, how patients with positive assay results should be further evaluated, and what the role of clinical judgment is in interpreting a positive result. These questions are distinctly tangential to our article, but our own view of these matters is as follows:

The burden of reducing the mortality and morbidity from ectopic pregnancy falls largely on the shoulders of busy clinicians, be they in resident training or private practice. The first responsibility of the clinician is to recognize the clinical circumstance that signals the need for immediate surgery and to bypass any type of testing, let alone the algorithmic plan of evaluation we have formulated.^{6,15,16} However, clinicians know only too well that in at least 70% of patients who turn out to have ectopic pregnancies, the clinical symptoms and signs

will overlap more or less inextricably with a host of other nonpregnant and pregnant conditions. Most also recognize intuitively, even if they cannot formally define it, that under such circumstances, as the positive predictive value of the clinical criteria they might apply to diagnose ectopic pregnancies increases, the proportion of all patients with ectopic pregnancies meeting these criteria will fall. Conversely, the worse the positive predictive value of those clinical criteria (up to an optimum), the greater the number of women with ectopic pregnancies that will meet those criteria, so that the number of missed cases will fall. This ineluctable fact follows from the direct relationship that exists between any "test's" true positive and false positive rates.³ It also explains why a good deal of guesswork is required to manage most ectopic pregnancies clinically and why many operative procedures that are not required become "necessary."

Assays of hCG, if properly used and interpreted, can remove most of the guesswork from the management of ectopic pregnancies precisely because they enable the index of suspicion for this disease to be raised without incurring a prohibitive false positive rate. The reason for this is partly because the majority of women suspected of but not having an ectopic pregnancy are not pregnant at all and partly because most false positive results (that is, when the pregnancy is not in the tube) can be identified by further evaluation.¹⁴ Clearly the false negative rate is of paramount clinical importance, given the nature of the condition, and takes precedence over what to do with a positive result. That is why the first question a clinician will ask is, "How certain can I be that a patient with a negative assay result does not have an ectopic pregnancy"; *this* is the question our paper addressed.

We agree that clinical judgment must always be exercised, and this applies to decisions about the further evaluation of patients with a positive assay result. But Landesman et al.'s suggestion that when "serum hCG values (are) more than 50 mIU/ml" the clinician will "establish a state of normal gestation by corroboration with clinical circumstances" simply does not reflect, in our opinion, what actually happens in practice at most major institutions in the United States. If an hCG assay has been requested, one should clearly be dealing with a patient who does not require immediate surgery. The decision about what to do with such a patient will nearly always be based on what a pelvic ultrasound scan shows. If sonography is nondiagnostic, a 48-hour delay to obtain a second hCG sample is worthwhile, since it is, in our experience, extremely unusual for the clinical situation to deteriorate over this time period, sparing the patient from an operative procedure. These issues have been previously discussed at length,^{1,2,6} so we will mention only our more recent findings which extend our subsequent observations and help to answer the authors' question about our assays false positive rate.

We are now able to define what constitutes a plateauing hCG titer (something we could only identify qualitatively hitherto,^{6,15,16}) and this finding is patho-

gnomonic for ectopic pregnancy. Our data shows that a rise or a fall in the serum hCG of 20% or less over a 48-hour period has so far had a 100% predictive value for an ectopic pregnancy (Kadar N, Romero R. Further observations on serial serum hCG patterns in ectopic pregnancies and abortions. Unpublished observations.) (Approximately 30% of ectopics will be associated with this pattern.) Titers of hCG that fall at a greater rate can be associated with either some type of abortion or an ectopic pregnancy, so curettage can be helpful. However, if the serum hCG is under 1000 to 1500 mIU/ml, and the serum hCG falls by more than 50% after 48 hours, there is a high chance that curettage will not yield chorionic villi. Not all of our patients falling into this category were subjected to a laparoscopy, so the possibility that some of them had resorbing ectopic pregnancies remains. However, we have had experience of six additional cases in which laparoscopy for failure to obtain villi at curettage, coupled with a positive hCG assay result, did not reveal the presence of an ectopic pregnancy. Private conversations with colleagues have revealed that they too have had personal experiences of such a situation.

Do such cases represent false positives in the sense implied by Landesman et al. (that is, a positive result in patients that were not pregnant at all) or simply the dissolution of the villi of a dead pregnancy and/or the failure to retrieve villi by curettage in very early pregnancies? We are aware of only two patients in the last 5 years of hCG testing in our institution who had a positive assay result and who had not had either confirmation of a pregnancy (sonographic, clinical, or histologic) or serial hCG determinations until the titers fell to undetectable levels (barring cases of trophoblastic diseases). We must therefore conclude that the false positive assay, with use of such a definition, is extremely small. These two patients were called to our attention by practicing colleagues, because they had persistently positive low hCG titers and therefore fell into the category of "discordant hCG results." The nature of this problem has been discussed in detail by Hussa et al.¹⁷

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Computerized tomography in cases of lipoid cell tumor causing virilization

To the Editors:

I am writing in reference to the article "Steroid secretion by a lipoid cell tumor causing virilization and its diagnosis with computerized tomography" by Fonseca et al. (*AM J OBSTET GYNECOL* 1985;153:797-8). I am perplexed by the emphasis in this article on the use of computerized tomography. It appears from the case report that the correct diagnosis and the indications for surgery did not involve the performance of a computerized tomographic scan. The findings of a dramatically elevated peripheral testosterone level and left ovarian enlargement on ultrasound were sufficient indication for laparotomy without other confirmatory, expensive radiographic studies. Indeed, had the left adnexal mass

been palpable on examination, even the ultrasound would have been redundant.

In addition, I must assume from the postoperative laboratory values that the treatment involved bilateral oophorectomy, presumably with hysterectomy and bilateral salpingectomy. If precise localization of the tumor site by computerized tomographic scan was able to pinpoint the tumor, what was the indication for a bilateral oophorectomy?

In a time when the watchword for American medicine is cost containment, we must all guard against the temptation to perform unnecessary and expensive diagnostic tests. A virilized patient with an elevated peripheral testosterone level and an adnexal mass does not need a computerized tomographic scan.

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Reply

To the Editors:

The diagnosis of virilizing ovarian tumor is frequently misleading; therefore it was a common procedure to perform a laparoscopy in order to establish the definitive diagnosis in those cases of severe virilization. We believe that the clinical and biochemical features associated with ovarian enlargement proved either on ultrasound or computerized tomographic scan are necessary to indicate the surgical approach. A small adnexal mass on pelvic examination may be deceiving, so that the confirmation by ultrasound or x-ray film is not redundant. The indication for bilateral oophorectomy in this case was the polycystic appearance of the contralateral ovary at the time of laparotomy.

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Prophylactic posterior culdoplasty

To the Editors:

In his recent article (AM J OBSTET GYNECOL 1985;153:135-9) Dr. Given very convincingly stressed the point that when a uterine prolapse is associated with a sizable enterocele, a well-executed posterior culdoplasty (either by McCall's or Torpin's technique) is a most important and integral part of the operation of vaginal hysterectomy. Unfortunately, the article has left the impression, which I must take issue with, that when the uterine prolapse is not associated with an overt enterocele, the performance of a prophylactic posterior culdoplasty at the time of the vaginal hysterectomy may be omitted.

The great master of abdominopelvic surgery, Victor Bonney, had taught that meticulous pelvic floor repair and vaginal vault suspension are most important integral parts of the operation of vaginal hysterectomy for uterine prolapse, even in the absence of an asso-

ciated overt enterocele.¹ This is done to prevent later posthysterectomy development of an enterocele and vaginal vault prolapse. Thus, residents in training should be taught to think first of the pelvic floor repair that is associated with vaginal hysterectomy for uterine prolapse even in the absence of an enterocele, instead of thinking first of the removal of the uterus. I prefer personally in the performance of prophylactic posterior culdoplasty the technique which has been described by McCall² and has so nicely been demonstrated in *TeLinde's Operative Gynecology*.³ I use a modification in which the strands of the previously placed retention sutures on the pedicles of the sacrouterine ligaments are used for the performance of the prophylactic posterior culdoplasty and the suspension of the vaginal vault.

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Reply

To the Editors:

In reply to Dr. Piura, I feel that I must indeed take issue with him in interpreting my article as giving the impression of a lack of interest in prophylactic culdoplasty at the time of hysterectomy.

The purpose was to give the technique and some long-term results with use of two types of culdoplasties in more difficult situations, that is, moderate to large enteroceles and even complete vagina eversion in patients wishing to maintain sexual function. In my article on page 136, it was stated "The McCall stitch was recommended by both the Mayo Clinic Group and in *TeLinde and Mattingly's Operative Gynecology* to be used prophylactically at the time of vaginal hysterectomy." Indeed, I frequently use these techniques at the time of vaginal hysterectomy and personally highly recommend them. However, it would be extremely difficult to prove their effectiveness without a large double-blind study. Waters¹ in 1956 reports one failure in 48 cases with wedge culdoplasty as compared to 14 recurrences of cystocele, enterocele, and/or vault prolapse in 210 vaginal hysterectomies without his technique. He also called attention to the use of posterior vault excision at the time of abdominal hysterectomy. Since almost half of the cases of posthysterectomy, enterocele, and/or prolapse of the vaginal vault follow abdominal hysterectomy, it would seem that some type of pro-

phylaxis is certainly important with either vaginal or abdominal hysterectomy.^{2,3} One of the pioneer vaginal surgeons of this country, N. Sproat Heaney, recommended a uterosacral approximation suture at the time of vaginal hysterectomy. This was pointed out by Hofmeister and used both for vaginal and abdominal hysterectomy in the discussion of Ranney's paper on enterocele.² Therefore prophylaxis is extremely important following either abdominal or vaginal hysterectomy and indeed needs to be emphasized.

The scope of our presentation was presented in the opening two paragraphs and obviously the use of these techniques for prophylaxis was not considered in this article. However, we do thank Dr. Piura for calling attention to the important addition of some type of culdoplasty at the time of hysterectomy.

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Immunohistochemical localization of human chorionic gonadotropin, placental lactogen, and pregnancy-specific globulin in early placental trophoblast: Implications for evaluating trophoblastic differentiation in germ cell neoplasms

To the Editors:

In a previous report (Tuttle SE, O'Toole RV, O'Shaughnessy RW, Zuspan FP. Immunohistochemical evaluation of human placental implantation: an initial study. *AM J OBSTET GYNECOL* 1985;153:238-44) we described our experience using antifactor VIII-related antigen (FVIII RAG) and anti-human chorionic gonadotropin (hCG) antibodies to localize corresponding antigen in placental implantation sites. Our main interest was to evaluate trophoblastic (presumably hCG positive) invasion of maternal blood vessels (FVIII RAG-positive) in the placental bed using these antibodies in the avidin-biotin complex immunoperoxidase technique. Placental bed specimens from varying lengths of gestation (8 weeks to term) were studied. Implantation sites from early pregnancy (8 to 17 weeks) were evaluated in hysterectomy specimens that were obtained for medical reasons.

One result of this initial study was of particular interest. Early placental sites contained many large mononucleated and multinucleated pleomorphic cells in the superficial placental bed which were seen migrating between maternal smooth muscle and decidual cells. These cells, which have been termed "intermediate trophoblast,"¹ have rather distinctive large, vesicular nuclei and eosinophilic cytoplasm. We found these cells to be essentially hCG negative on immunostaining, with the exception of occasional hCG-positive trophoblastic cells which were seen in close proximity to maternal blood vessels.

Since these cells were found to be hCG negative, we could not establish their trophoblastic histogenesis. A previous report had described intermediate trophoblasts with strong positive immunostaining for HPL and focal staining for hCG.¹ We have therefore extended our observations of these early implantation sites to include immunostaining with primary antibodies (all obtained commercially from DAKO Corp., Santa Barbara, CA) directed against placental hormones, human placental lactogen and pregnancy-specific globulin, in addition to hCG. We have confirmed that the intermediate trophoblast shows strong immunostaining for human placental lactogen, but essentially negative staining for pregnancy-specific globulin and hCG. The human placental lactogen-positive intermediate trophoblast was localized to the superficial placental bed in each specimen obtained early (8 to 17 weeks) in gestation. Human placental lactogen immunostaining clearly helped in distinguishing mononucleated and multinucleated intermediate trophoblast from decidual and smooth muscle cells of the placental bed which were human placental lactogen, hCG and pregnancy-specific globulin negative. Immunostaining patterns for hCG and pregnancy-specific globulin were very similar in these early placental sites. Syncytiotrophoblast lining chorionic villi were strongly hCG and pregnancy-specific globulin positive, while human placental lactogen was only weakly positive in the syncytiotrophoblast.

This pattern of pregnancy-related hormone production by trophoblast of varying morphology and location in the placental bed implies a functional complexity of placental trophoblast which needs further evaluation. The mechanisms that control the synthesis and secretion of these placental hormones are presently unclear. It appears that the invasive intermediate trophoblast synthesizes human placental lactogen in preference to hCG but that trophoblast within vessel walls or in lumina of maternal blood vessels preferentially synthesize hCG.

In addition, these immunostaining results on trophoblastic elements in the normal placental site have significant implications for the identification of trophoblastic differentiation in the neoplastic state. The existence of human placental lactogen-positive and hCG- and pregnancy-specific globulin-negative trophoblastic elements in the normal placental bed sug-

gests that the evaluation of germ cell neoplasms may need to include human placental lactogen immunostaining in addition to staining for hCG. Our results imply that the addition of human placental lactogen immunostaining could significantly increase sensitivity in detecting trophoblastic differentiation in a variety of germ cell neoplasms.

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Elective repeat cesarean delivery and persistent pulmonary hypertension of the newborn

To the Editors:

We read with interest the report by Heritage and Cunningham on the association of elective cesarean section and persistent pulmonary hypertension of the newborn (*AM J OBSTET GYNECOL* 1985;152:627-9). They were able to provide convincing clinical evidence to substantiate this association, but appropriate etiologic explanation has not been given.

In this respect it should be noted that clinical, laboratory, and morphologic evidence has been accumulated which indicates that the reduced level of circulating prostaglandins may be involved in the control of pulmonary circulation of the neonate by demonstrating high incidence of the persistence of fetal circulation syndrome in newborn infants born to mothers receiving prostaglandin synthetase inhibitor therapy.¹⁻⁴

Moreover, it has also been shown that plasma prostaglandin E and $F_{2\alpha}$ levels are significantly lower in the cord blood of infants delivered by elective cesarean section than of those delivered vaginally.^{5,6}

In this context it is of interest that the high pulmonary vascular resistance induced by hypoxia and hypercapnia could be controlled with prostacyclin in neonatal swine.⁷

On the basis of these observations we have suggested that the low rate of endogenous prostaglandin production during elective cesarean section might be responsible, at least in part, for the high pulmonary vascular resistance, the pulmonary hypoperfusion, the right-to-left shunting, and the subsequent development of neonatal respiratory distress syndrome.⁸

The findings presented by Heritage and Cunningham can be regarded as strongly supporting our

hypothesis derived from convincing but indirect clinical evidences.

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Reply

To the Editors:

In their letter Sulyok and Csaba suggest an explanation for the association of elective cesarean delivery and neonatal respiratory distress syndrome. They argue that endogenous prostaglandin production is decreased during elective cesarean delivery with a resulting increase of pulmonary vasculature resistance leading to neonatal respiratory distress syndrome. Their argument is based on indirect evidence drawn from the literature and their experience with preterm neonates who developed respiratory distress syndrome.¹ Our observations dealt with the association of elective repeat cesarean delivery and persistent pulmonary hypertension in the term neonate; none of our cases exhibited findings consistent with neonatal respiratory distress syndrome. Since no clear etiologic explanation of persistent pulmonary hypertension of the newborn currently exists, we chose not to speculate on the relationship to elective cesarean deliveries.

Others have reported decreased prostaglandin production during elective cesarean delivery while noting that the presence of labor tends to prevent increased pulmonary vascular resistance.^{2,3} Because 10 of our 12

cases did not experience labor, we are inclined to agree with the reasoning of Sulyok and Csaba that our report supports their hypothesis of increased pulmonary vasculature resistance following elective cesarean delivery. But until the effects of prostaglandins on fetal and neonatal pulmonary circulation are fully documented, the association of the absence of labor, persistent neonatal pulmonary hypertension, and decreased prostaglandin production remains speculative.

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- Sudden Infant Death.** Jean Golding, Sylvia Limerick, and Aidan Macfarlane. 272 pages. Seattle, 1986, University of Washington Press. \$25.00.

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

1st World Symposium on Computers in the Care of the Mother, Fetus and Newborn, March 8-11, 1978, Vienna, Austria. Deadline for submission of abstracts is November 29, 1986. For further information please contact: Robert J. Sokol, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, Wayne State University, Hutzel Hospital, 4707 St. Antoine, Detroit, MI 48201. Tel.: (313) 745-7530.

Advanced Perinatal Ultrasound Seminar, co-sponsored by the Department of Radiology, Orlando Regional Medical Center, and the Center for Medical Ultrasound, Bowman Gray School of Medicine, will be held on February 26-28, 1987, at the Buena Vista Palace Hotel, Lake Buena Vista, Florida. For information contact one of the following: Lennard D. Greenbaum, M.D., Chief, Section of Diagnostic Ultrasound, Orlando Regional Medical Center, 1416 South Kuhl Ave., Orlando, FL 32806. Tel.: (305) 841-5229. Frederick W. Kremkau, Ph.D., Director, Center for Medical Ultrasound, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103. Tel.: (919) 748-4505.

Problem Solving in Gynecologic Endocrinology and Infertility, December 12-13, 1986, The Knickerbocker Hotel, Chicago, Illinois. 10 Hours, Category I, 10 Cognates, Formal Learning, ACOG. For information contact: The University of Chicago, Office of Continuing Medical Education, 5841 Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 962-1056.

Eighteenth Memphis Conference on the Mother, Fetus and Newborn, September 25-26, 1986, The Peabody Hotel, Memphis, Tennessee. Sponsored by The University of Tennessee College of Medicine. For further information contact: Jean Bryan, Office of Continuing Medical Education, 800 Madison Ave., Memphis, TN 38163. Tel.: (901) 528-5547.

Eighth Annual Obstetrical Office Ultrasound Physician's Workshop, October 12-15, 1986, Gatlinburg, Tennessee. Presented by The University of Tennessee College of Medicine, Knoxville Unit, Department of OB/GYN. For further information contact: Abby Hall, Department of Continuing Medical Education, D-116, 1924 Alcoa Highway, Knoxville, TN 37920. Tel.: (615) 544-9190.

Sixth Annual Smoky Mountain Seminar in OB/GYN, October 6-8, 1986, Park Vista Hotel, Gatlinburg, Tennessee. Presented by The University of Tennessee College of Medicine, Knoxville Unit, Department of OB/GYN. For further information contact: Abby Hall, Department of Continuing Medical Education, D-116, 1924 Alcoa Highway, Knoxville, TN 37920. Tel.: (615) 544-9190.

University of Vermont Postgraduate Course in Obstetrics and Gynecology, Radisson Hotel Burlington, Vermont, October 6-8, 1986. For further information contact: Maureen Hanagan, University of Vermont College of Medicine, Burlington, VT 05405. Tel.: (802) 656-2292.

GYNECOLOGIC ENDOCRINOLOGIST

Allegheny General Hospital, is a 726-bed acute care teaching hospital, located in Pittsburgh, PA. We are currently seeking a board eligible/board certified individual to join a multi-specialty group practice. University affiliation and research opportunities available. Please send resume to: Virginia Opiare, Vice President.

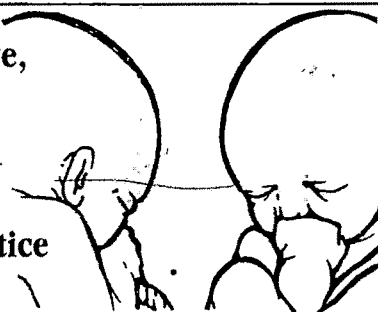


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Bronson Methodist Hospital, a regional perinatal center in southwest Michigan, seeks a board certified/eligible obstetrician-gynecologist to join its medical staff of 23 board certified obstetricians, a maternal referral service under perinatologist direction, a 45-bed neonatal intensive care unit staffed by six neonatologists, a dedicated high risk antepartum unit, complete labor/delivery, postpartum and nursery facilities and more.

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Bronson Methodist Hospital
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Obstetrician-Gynecologist

needed to join seven person Department in 165 multi-specialty clinic; clinic and adjacent 288-bed hospital are located in Big 10 University community of 100,000; teaching positions are available in medical school for those with academic inclination; liberal fringe benefits and salary lead to equal ownership. Write including CV to:

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The Department of Obstetrics & Gynecology of the College of Physicians & Surgeons and The Presbyterian Hospital

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Survey of the major clinical and pathological problems pertaining to the female reproductive system. Designed for practicing clinicians and for candidates for the Board Examinations. Emphasis is placed on the diagnosis and management of obstetrical and gynecological disorders, including infertility, endocrine problems and malignant diseases. A review of gynecologic pathology is included. Modern advances in basic knowledge and application to clinical practice is stressed. The course is divided as follows: gynecological oncology, perinatal medicine, fetal and maternal medicine, reproductive endocrinology and gynecological surgery and infertility. The **Second Annual Raymond L. Vande Wiele Memorial Lecture** will be presented by Mortimer G. Rosen, M.D., on Wednesday, October 29th, at 1:30 p.m.

Contact: Elizabeth C. Gerst, Ph.D., Assistant Dean for Continuing Education, 630 West 168th Street, N.Y., N.Y. 10032; telephone (212) 305-3682.

Genetics, Endocrinology, and Developmental Biology Medical Staff Fellowships

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National Institutes of Health

The National Institute of Child Health and Human Development of the National Institutes of Health is offering a limited number of three-year Medical Staff Fellowship positions to candidates of the highest caliber. Fellowships begin July 1, 1988 and 1989. Positions are available for physicians who have completed at least two years of training in Internal Medicine, Pediatrics, or Obstetrics and Gynecology. Other medical specialty credentials will be considered on a case by case basis.

The training program consists either of one clinical year and two years of laboratory-based research (satisfying the requirements for the Subspecialty Boards in Human Genetics, Adult or Pediatric Endocrinology, or Gynecologic Endocrinology), or three years of laboratory research exclusively. The endocrinology fellowships are integrated with the NIH Interinstitute Endocrinology Fellowship Program, and the human genetics fellowships are integrated with the NIH Interinstitute Medical Genetics Program. The gynecologic-endocrinology fellowship is integrated with programs at the Walter Reed Hospital and the National Naval Medical Center.

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The major emphasis of the NICHD Intramural Research Program is on basic and clinically applied research in the broad area of developmental biology. Areas presently being examined include developmental endocrinology, reproductive biology, cellular differentiation, human genetics and in-born metabolic errors, molecular endocrinology, developmental and molecular immunity, developmental neurobiology, molecular genetics (bacteria, virus, yeast, drosophila, and animal cell), pharmacogenetics, growth factors, neurochemistry and neuro-immunology, theoretical and physical biology, cell biology (especially receptors and organelles) and comparative ethology.

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Interested candidates should send their curriculum vitae to (or request further information from):
Arthur S. Levine, M.D., Scientific Director
National Institute of Child Health and Human Development
National Institutes of Health, Building 31, Room 2A50
Bethesda, Maryland 20892; (301) 496-2133.

Deadline for applications is November 15, 1986.



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GENERAL OBSTETRICIAN AND GYNECOLOGIST

This combined appointment offers an excellent practice opportunity within a thriving and up to date community hospital located in a lovely university community within the Heartland of Central New York's finger lakes region.

The Hospital maintains close connections to SUNY, Health Science Center in nearby Syracuse. An academic appointment will be offered through the Department of Obstetrics and Gynecology, SUNY, Syracuse.

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A/C 607-734-4191

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216 S. Kingshighway
St. Louis, MO 63110

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Pennsylvania Hospital is an affiliate of the University of Pennsylvania and is an affirmative action/equal opportunity employer.

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Department of Obstetrics and Gynecology
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8th & Spruce Streets
Philadelphia, PA 19107

CHIEF DEPARTMENT OF OBSTETRICS & GYNECOLOGY

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OB/GYN Chief Search Committee
c/o Richard D'Aquila, Vice President
Mount Sinai Hospital
500 Blue Hills Avenue
Hartford, Connecticut 06112
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or call (415) 929-4845

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Department of Obstetrics-Gynecology
Lubbock, Texas 79430

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The Department of Obstetrics and Gynecology, Medical College of Virginia, Virginia Commonwealth University is recruiting an Assistant Professor to work in the Maternal Fetal Medicine section. This is a full time faculty position requiring Board certification or eligibility, as well as certification or eligibility in Maternal Fetal Medicine. The responsibilities include undergraduate and graduate medical teaching, patient care and clinical or laboratory research.

If interested, please submit a c.v. by 10/31/86 to:

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Professor and Chairman
Department of Ob/Gyn, Box 34
Medical College of Virginia
Richmond, Virginia 23298

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OB/GYN GENERALIST

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Pennsylvania Hospital is an affiliate of the University of Pennsylvania and is an affirmative action/equal opportunity employer.

Interested candidates should send a curriculum vitae and letter to:

Linda K. Dunn, M.D.
Department of Obstetrics and Gynecology
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

OB/GYN

Board Certified or eligible OB / GYN sought to join group practice of 1 OB/GYN, 2 FPs, & 1 FNP. Located at foot of Sierra-Nevadas. Excellent salary, health & dental insurance. Malpractice insurance paid. Contact:

Oscar Velasco, M.D.
Medical Director
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1107 W. Poplar
Porterville, CA 93257
or call (209) 781-7242

PERINATOLOGIST

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(206) 842-5248

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The applicant must be board eligible or certified for both general obstetrics and gynecology and gynecologic oncology and be eligible for Georgia license.

Academic rank dependent on previous experience. Position available July 1, 1987. Please forward CV to:

O. Eduardo Talledo, M.D.

Professor, Obstetric and Gynecology
Director, Section of Gynecologic
Oncology

Medical College of Georgia
Augusta, Georgia 30912

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Department of Obstetrics & Gynecology
Jefferson Medical College
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Philadelphia, PA**

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Equal Opportunity Employer

MATERNAL-FETAL MEDICINE FACULTY POSITION

The Division of Maternal-Fetal Medicine of the Department of Obstetrics and Gynecology at the University of Florida College of Medicine in Gainesville, Florida has an opening for a full-time faculty position at the Assistant to Professor rank.

Board eligibility or certification in Maternal-Fetal Medicine is required and training or experience in obstetric ultrasound is desirable.

Salary and rank commensurate with training and experience. Application Deadline: October 17, 1986. Anticipated Starting Date: January 1, 1987.

Send curriculum vitae to:

Amelia C. Cruz, M.D.

Department of Obstetrics and Gynecology
J-294; JHMHSC

University of Florida College of Medicine
Gainesville, Florida 32610

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FACULTY POSITIONS

The Department of Obstetrics and Gynecology at Washington University School of Medicine will assume responsibility for staffing and supervising the Obstetrical-Gynecological Service at St. Louis Regional Medical Center and announces, in conjunction with that institution, the following available faculty positions:

- Chief of Obstetrical-Gynecological Service (Board eligibility or certification in Maternal-Fetal Medicine) at the Associate Professor or Professor level.
- Associate Chief of Obstetrical-Gynecological Service (demonstrated credentials in gynecology) at the Assistant Professor or Associate Professor level.
- Specialists in Maternal-Fetal Medicine (Board eligibility or certification in Maternal-Fetal Medicine) at the Assistant Professor or Associate Professor level.

As of 1987, residents and students in the Washington University teaching program will be evenly distributed among our three teaching units: Barnes Hospital, The Jewish Hospital of St. Louis, and St. Louis Regional Medical Center.

Interested parties should contact:

James C. Warren, M.D., Ph.D.

Professor and Head

Department of Obstetrics and Gynecology
Washington University School of Medicine
4911 Barnes Hospital Plaza
St. Louis, Missouri 63110

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POSITION AVAILABLE— REPRODUCTIVE ENDOCRINOLOGIST

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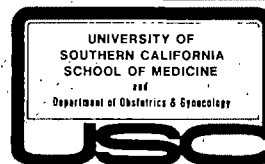
Successful candidate will have University funding for resident teaching, with ample opportunities for private practice development. Expansive practice opportunity developed and awaiting appropriate candidate. Experienced microsurgeon and interest in continuing development on an in-vitro fertilization program required. Opportunities for scientific and clinical research related to reproductive endocrinology.

Reply to:

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PERINATAL MEDICINE

February 7-13, 1987

Royal Lahaina Hotel, Maui, Hawaii

This course covers a wide spectrum of reproductive medicine but particularly emphasizes the outcome and events associated with the perinatal period. Areas of particular emphasis will relate to the ultrasonic technology as it is currently applied with future applications considered. A number of clinically pertinent areas will be covered with special emphasis given to the management aspects of frequent obstetrical problems. The course structure is designed to permit lengthy open question and answer sessions daily. Interactive sessions during breakfast will permit the participants to address their particular problem cases to a faculty person and other attendees.

Tuition: \$525
Credit: 23 AMA/CMA Category I hours
Contact: Associate Dean, USC School of Medicine, Postgraduate Division, KAM 307, 1975 Zonal Avenue, Los Angeles, CA 90033 (213) 224-7051.



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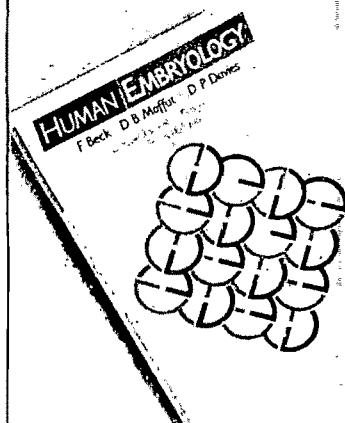
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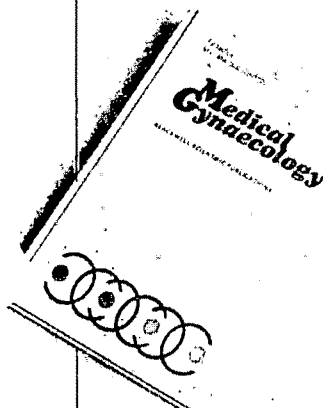
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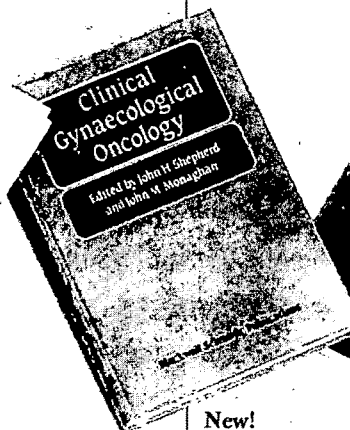
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Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 0.05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS: An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age.

CEREBROVASCULAR DISORDERS: In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 5.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders in both users and nonusers of oral contraceptives increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 women (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth; with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (leg, thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. Feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. **2. OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. **3. CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogens in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential, in all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. **4. HEPATIC TUMORS:** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral

Enhanced patient compliance

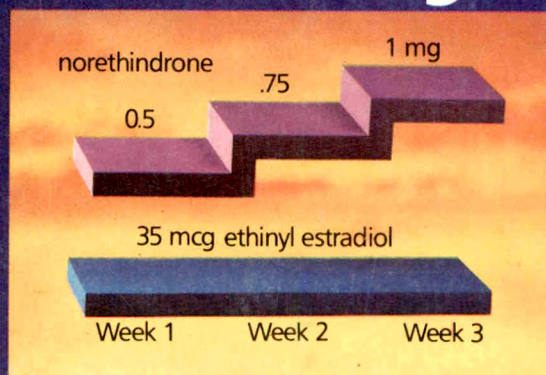
Tablets

contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. **5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones. The use of female sex hormones in pregnant women has been reported with the use of sex hormones. The risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion) of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. **6. GALBLADDER DISEASE:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. **7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS:** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. **8. ELEVATED BLOOD PRESSURE:** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. **9. HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. **10. BLEEDING IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation possibly prolonged, may also occur in women without previous irregularities. **11. ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **12. BREAST FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers taking these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS:** **General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that a woman becomes pregnant shortly after stopping oral contraceptives, she may have a decreased level of folate and be subject to complications attributable to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. **10.** Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalene retention; b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin; c. increased norepinephrine-induced platelet aggregability; d. increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; d. Decreased pregnandiol excretion; e. Reduced response to metoprolol test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concurrent use of oral contraceptives and certain anticonvulsants (phenytoin, carbamazepine, phenobarbital, sodium, ampicillin, griseofulvin, and tetracycline). **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS:** See CONTRAINDICATIONS AND WARNINGS. **ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis, Pulmonary embolism, Coronary thrombosis, Cerebral thrombosis, Cerebral hemorrhage, Hypertension, Gallbladder disease, Liver tumors, Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis, Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs infrequently, usually within the first cycle. Other reactions, such as anorexia, loss of appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, change in scalp hair, Erythema multiforme, Erythema nodosum, Hemorrhagic eruption, Vaginitis, Porphyria, Impaired renal function, Hemolytic uremic syndrome. **OVERDOSEAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.



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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see complete Prescribing Information, a summary of which appears on the preceding page.

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